Atomic Layer Deposition:
A Novel Strategy to Remineralize Calcium Hydroxyapatite Matrix

BY
BELA DEEPAK JOSHI

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THESIS
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Defense Committee
Dr. Christos Takoudis, Chair and Advisor
Dr. Cortino Sukotjo, College of Dentistry
Dr. Anne George, College of Dentistry
Dedication

I would like to dedicate this piece of work to my family and friends for their constant support. I would particularly like to dedicate my thesis to my parents and my aunt. Their unconditional support and inspiration made this work possible.
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<td>ALD</td>
<td>Atomic Layer Deposition</td>
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<tr>
<td>CVD</td>
<td>Chemical Vapor Deposition</td>
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<tr>
<td>PLD</td>
<td>Physical Vapor Deposition</td>
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<td>HAp</td>
<td>Hydroxyapatite</td>
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<tr>
<td>ITD</td>
<td>Intertubular Dentin</td>
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<tr>
<td>PTD</td>
<td>Peritubular Dentin</td>
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<tr>
<td>CaP</td>
<td>Calcium Phosphate</td>
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<tr>
<td>BAG</td>
<td>Bioactive Glass</td>
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<tr>
<td>RMS</td>
<td>Remineralizing Solution</td>
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<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>EDS</td>
<td>Energy Dispersive X-ray Spectroscopy</td>
</tr>
<tr>
<td>TDEAT</td>
<td>Tetrakis(diethylamino)titanium(IV)</td>
</tr>
<tr>
<td>aq</td>
<td>aqueous</td>
</tr>
<tr>
<td>$D_o$</td>
<td>Original Diffusivity</td>
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<tr>
<td>$D_{eff}$</td>
<td>Effective Diffusivity</td>
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Summary

The following research work aimed to investigate the potential of atomic layer deposition technique to coat a high aspect ratio biomaterial i.e. hydroxyapatite with TiO\textsubscript{2} nanofilm. Low temperature (100 °C) atomic layer deposition was performed with long exposure time of precursor (TDEAT) along with O\textsubscript{3} as an oxidizer and N\textsubscript{2} as the purging gas. The primary focus was to achieve infiltration of porous substrate with TiO\textsubscript{2} by coating the entire exposed area using atomic layer deposition technique. Another aim of the study was to examine the ability of as-deposited TiO\textsubscript{2} nanofilm to nucleate calcium phosphate crystals in biomimetic remineralizing solution (in vitro conditions) and therefore to remineralize the demineralized hydroxyapatite matrix. The analysis of nucleation and crystal growth was performed at 4 different times. The control samples and test samples (with as-deposited TiO\textsubscript{2} nanofilm) were analyzed after 0 week (immediately after ALD), 1, 2 and 3 weeks immersion period. Scanning electron microscopy and Energy dispersive x-ray spectroscopy methods were used for morphological and chemical analysis.

Results of the experiments showed that low temperature atomic layer deposition (ALD) was able to grow uniform TiO\textsubscript{2} nanofilm on hydroxyapatite surface suggesting potential nucleating sites on the substrate surface. The porous hydroxyapatite substrate was infiltrated with TiO\textsubscript{2} throughout its depth with uniform Ti content values obtained from EDS analysis suggesting uniform ALD process. Also, clear morphological difference was seen between the surface control and as-deposited test samples. Ca and P nucleation was observed after a period of 1 week. Larger crystal clusters were seen on the surface test samples with as-deposited TiO\textsubscript{2} after a period of 2 weeks whereas control samples with no prior ALD treatment showed no crystal growth. Week 3 analysis showed larger area covered with newly nucleated CaP crystals suggesting ability of as-
deposited TiO$_2$ film to nucleate Calcium phosphate from biomimetic remineralizing solution at a pH above 6.5. The cross sectional analysis showed no appreciable increase in Ca and P content with no morphological difference between control and test samples over a period of 3 weeks. The diffusivity is supposed to be affected mainly due to viscosity barriers offered by remineralizing solution. The atomic layer deposition to coat hydroxyapatite with TiO$_2$ showed promising results in order to nucleate Ca and P crystals, however, the future work can be done to achieve appreciable CaP nucleation throughout the hydroxyapatite matrix i.e. on the tubular region (cross sectional area) as well. Possible ways to directly coat demineralized hydroxyapatite substrate by CaP using ALD based on available literature have also been suggested.
Chapter 1

Introduction

1.1 Atomic Layer Deposition

Thin Film is defined as a very thin layer of material usually ranging from angstroms to few micrometers in thickness. Deposition of a thin film of a material onto a substrate of any kind is known as ‘Thin Film Deposition’. Thin film coating has applications in various fields such as semiconductors, batteries, solar industry, bone implants etc. [1-4]. Thin film deposition can be broadly classified into 2 main categories which are physical deposition and chemical deposition. Advanced vapor deposition techniques resulting in chemical deposition are mainly chemical vapor deposition (CVD) and atomic layer deposition (ALD) [5]. ALD is the most advanced version of the traditional CVD process [6]. These chemical vapor deposition techniques used to produce thin films on the substrate surface have emerging applications in various fields and research is going on for the development of advanced applications.

In case of chemical vapor deposition, the substrate is heated to the desired temperature and a mixture of gases is allowed to flow over the surface of the substrate (Figure 1.1). The heated surface accelerates the reaction between gaseous species resulting into the thin film formation on the surface. In an ideal situation, reacting gases will not react until they reach the heated surface [7, 8]. But due to the gas phase conduction of heat, mixture of gases may react before reaching the surface resulting into non-uniform and non-conformal coating. These homogeneous gas phase reactions may result in formation of particles which get incorporated in the film responsible for non-conformal film growth [9]. Usually very low pressure/ vacuum conditions are desired for the uniform growth of thin film layer [9, 10].
Figure 1.1: Schematic diagram of conventional CVD process. Heated substrate is exposed to a stream of reactants which allows surface reaction after they reach the surface. Homogeneous gas phase reactions can also possibly occur.

ALD is an advanced variant or a new subset of CVD technique. In ALD, instead of mixture of the gases, gaseous reactants are exposed to the substrate surface in separate steps. The ALD steps can be summarized as follows (Figure 1.2):

Step 1: Exposure of the first precursor (reactant A)

Step 2: Purge to remove excess unreacted reactant and by-products from the reaction chamber

Step 3: Exposure to the second precursor (reactant B)

Step 4: Purge to remove excess unreacted reactant and by-products from the reaction chamber
These 4 steps constitute 1 ALD cycle and these cycles are repeated in order to produce thin film of desired thickness.

Figure 1.2: Schematic diagram of ALD process showing 4 steps that constitute an ALD cycle

After the substrate is exposed to the first reacting gaseous reactant, it gets adsorbed on the exposed surface of the substrate resulting in the formation of monolayer of the first reactant. Step 1 is continued for sufficient amount of time to allow the whole surface to get covered by this
monolayer. This monolayer remains adsorbed on the surface after the reaction chamber is evacuated by the purging gas. In the next step, another reactant is allowed to flow over the substrate when reaction occurs between adsorbed monolayer and 2\textsuperscript{nd} reactant forming desired film. This cycle is repeated in order to achieve a thin film of desired thickness. Formation of the monolayer after exposure to precursor gases is responsible for self-limiting mechanism making controlled film growth possible in case of ALD [8]. As the reacting precursors are brought to the substrate in 2 different steps, the homogeneous gas phase reaction is completely avoided. Consequently, pinhole free coating with desired composition is expected with ALD. Also, as the film growth totally depends on monolayer adsorption on the substrate surface, thickness of the film on the plane surface does not vary from the corners of the surface. This provides additional advantage of more conformal and uniform thin film deposition [11]. Moreover, thin film grown using ALD does not alter the original shape of the substrate. The substrate temperature as well as flow uniformity of the reacting gases do not influence the film formation. The concentration of the flowing gases is also not of critical importance. As long as the surface receives sufficient amount of precursors to produce a monolayer, the uniform films will continue to grow on a substrate with any topography. Hence, ALD is suitable for large and complex structured surface when uniform thin film is desired [11]. By tuning the ALD reaction parameters (temperature of the precursors, number of cycles, pressure conditions and exposure and purge times) desired film thickness can be achieved on the uneven surfaces. As the film growth depends on the adsorbed monolayer, ALD process has a disadvantage of low film growth rate. It is experimentally verified that film growth rate in case of ALD is usually one or two orders of magnitude less than the corresponding CVD or other physical vapor deposition processes.
ALD on substrates with high aspect ratio is a topic of research interest [12, 13]. ALD is used to coat substrates with deep trenches or porous structures. Here, Due to conformal and uniform film growth, not only the top surface but also the exposed area in the trenches or pores is expected to get coated with the desired thin film. The entire area to be coated is to be exposed to the incoming precursors and purge gas in order to form the complete monolayer which is critical for conformal film growth.

1.2. Atomic Layer Deposition on Biomaterials

Atomic layer deposition on biomaterials is a newly emerging field of research interest. Due to the temperature sensitivity of biomaterials (usually protein and peptide based or biomineralized substrates) ALD is not widely practiced on these kind of substrates [14]. Protein based nanostructures such as Tobacco Mosaic Virus, Ferritin [15]as well as natural fibers such as cotton fibers, sea mouse bristles [14, 16] are the substrates on which ALD of TiO$_2$ or Al$_2$O$_3$ has been tried. The porous substrates were tried to infiltrate with metal oxide using ALD.

Recently, Inner shell membranes (ISM) of avian eggshells were used as templates to prepare ZnO and TiO$_2$ membranes using low temperature atomic layer deposition [17]. Both the coated membranes exhibited mechanical flexibility and thermal stability. Another study was performed on spider dragline silk. Infiltration of the metals Zinc (Zn), Aluminum (Al) and Titanium (Ti) into the spider dragline silk resulted in improved toughness of the substrate [18]. Collagen membranes were in infiltrated with the same metals using ALD (multiple pulsed vapor phase infiltration mode) [19]. Mechanical stability was found to increase by a factor of 3 than that of untreated collagen membrane. Thus, ALD on various biomaterials has been tried for applications mainly focused on improving mechanical strength of the original substrate. Apart
from that, ALD and CVD modes were compared for the purpose of infiltration on metal oxide into the biomaterials [20]. In case of spider dragline silk and collagen membrane, the ALD mode was efficient with respect to infiltration of the substrate with metal oxide and mechanical stability of the substrate. Thermal stability of the biomaterial is a factor of concern in case of biological substrates due to presence of proteinaceous substances which are prone to chemical changes affecting original composition and physicochemical properties of the substrates. ALD has attracted scientists due to its ability to form pinhole free coatings at desired low temperature [21].

1.3 Dental Caries

Dental caries or tooth decay is a chronic disease affecting people of all ages worldwide [22]. This is responsible for producing tooth cavities demanding expensive dental treatment. It is important to understand the structure of human tooth (Figure 1.3) to study dental caries and its causes.

1.3.1. Structure of tooth

Enamel, dentine and cementum are the hard tissues of normal tooth which contain hydroxyapatite crystals as their mineral phase. These 3 parts are described in this section.

![Figure 1.3: Structure of human tooth][23]
1.3.1.1 Enamel

Enamel is the hardest and highly mineralized substance in human body [24]. 96% of the enamel is made up of mineral phase which is made up of “Hydroxyapatite Crystals” (HAp). Remaining 3% is water and 1% is made up of organic matter. Hardness of tooth enamel is 5 on Mohs scale. High mineral content of enamel accounts for not only its strength but also brittleness.

Basic unit forming tooth enamel is “HAp crystallites”. Lattice arrangement of HAp crystals has been found to be hexagonal with lattice parameters a=9.4412 Å and c=6.878 Å [25]. HAp crystallites unite to form nanorods which are around 10 nm in diameter and 100 µm in length. These nanorods form clusters of around 1000 crystallites to give enamel rods or prisms. This is the main unit of enamel rod. These enamel rods or prisms form tooth enamel. Enamel prisms are around 5 µm in diameter and few mm in length [26].

![Enamel Rod Diagram](image)

**Figure 1.4:** Structure of enamel showing arrangement of enamel rods (keyhole)

Figure 1.4 shows the normal structure of enamel rod. This rods have head and tail and are in a shape of a “keyhole” [26]. The long axis of this enamel rod is perpendicular to the underlying dentin. Their orientation is not the same throughout the tooth. The enamel rods near the cemento-enamel junction (CEJ) tilt slightly towards the root of the tooth. The orientation of HAp crystallites in the rods is also peculiar [27]. Minute spaces, called pores exist where crystals do not form
between rods. They are responsible for enamel’s permeability. Thus, most of the pores in the enamel are parallel to dentin-enamel junction [28].

1.3.1.2. Dentin

Dentin is the second hardest tooth hard tissue. It contains 70% mineral phase, 20% organic material and 10% water by weight [25]. As it is less hard than the enamel, its brittleness is also lower than that of the enamel. Its hardness is 3-4 on the Mohs scale. Dentin consists of multiple dentinal tubules which are perpendicular to the enamel-dentin junction.

Representative SEM image of fractured dentine surface showing the ITD (intertubular dentin) and PTD (peritubular dentin) [29]. Tubules are perpendicular to the surface. Mean diameter of dentin tubule is about 1.21 ± 0.08 µm and an average width of PTD is reported to be around 0.80 ± 0.09 µm. The distance between 2 tubules is 5-10 µm. Thus, dentin is a porous structure which comprises of dentin tubules. Figure 1.5 shows the SEM image of top surface of the dentin and Figure 1.6 shows a distinct tubular opening.
Figure 1.5: SEM image of Top surface of the human dentin showing dentinal pores which are openings of the dentinal tubules

Figure 1.6: SEM image of the top surface of dentin with high magnification showing a dentin pore

1.3.1.3 Cementum

Cementum is slightly softer than dentin and consists of about 45% inorganic material (HAp) by weight and 33% organic matter and 22% water by weight. The organic portion is
composed primarily of collagen and protein polysaccharides [30]. Hardness of cementum is 2-3 on Mohs scale.

1.3.2 Chemical Phenomena [31-33]

Biofilm present on the tooth surface contains fermentable carbohydrates which are prone to bacterial attack. The acid producing bacteria ferment these carbohydrates and give away by products such as lactic acid, formic acid and propionic acid. These acids penetrate into the hard tissue matrix of tooth degrading structure of hydroxyapatite. These acids can dissolve the calcium phosphate minerals i.e. inorganic content of dentin at acidic pH which is termed as demineralization of tooth.

![Diagrammatic representation of demineralization and remineralization processes](image)

*Figure 1.7: Diagrammatic representation of demineralization and remineralization processes*
Dental caries or tooth decay results from interaction between saliva, acid producing bacteria as well as tooth minerals. Human saliva contains minerals (Ca and P) which can replenish the lost minerals from hydroxyapatite matrix of tooth. Thus, there is an equilibrium reaction maintained between loss of minerals from tooth and build up by deposition of minerals from the saliva solution [34]. The failure to maintain the balance between these two simultaneously occurring phenomena results in tooth decay and cavity formation in the tooth. Initially the loss of minerals takes place from enamel crown and eventually it affects dentin hard tissue matrix. Dental caries is preventable in its early stages [35].

1.3.3 Previous Studies Involving Tissue Regeneration Strategy

Strategies to cure dental caries is an area of tremendous research interest. Different strategies have been used to restore the lost mineral matrix of tooth. During early stages of dental caries, the HAp mineral matrix is not completely devoid of the mineral phase. Thus, it has potential to attract the mineral ions when it comes in contact with the solution containing those. Tissue regeneration strategy is building up the lost hard tissue matrix of tooth HAp [36]. Different techniques have been previously used to reconstruct the mineral phase in collagen matrix of tooth HAp out of which few of them showed promising results.
Ari-Pekka Forsback et. al. conducted an in vitro study of mineralization of dentin using bioactive glass S53P4 (BAG) particles to produce CaP layer on the surface of dentin [37]. BAG and regular commercial glass (CG) were used for comparison and immersed in remineralizing solution to investigate the potential of glass particles to attract Ca and P minerals. The dentin substrates were immersed in remineralizing solution containing these glass particles (diameter >50 nm). Dissolved silica from the glass was adsorbed on the dentin substrate responsible for attracting minerals. Dentin substrate infiltrated with BAG showed undesired demineralization after a period of 2 weeks.

On the similar lines, Meret Vollenweider et. al. tried ultrafine bioglass particles (diameters in the range of 20 to 50 nm) [38]. Dentin bars were treated with ultrafine nanoparticles immersed in remineralizing solution (RMS) for a period of 30 days. Rapid increase in mineral content of demineralized dentin was observed. But it showed no mechanical reconstitution. This was attributed to imperfect arrangement of newly deposited material.

Another strategy was layer by layer deposition of CaP on the demineralized collagen matrix using Ca(OH)₂ suspension and NaH₂PO₄ solution [39]. Layer by layer deposition was done by dipping the dentin substrate in these solution alternatively. Remineralization potential and adhesive capacity of this deposited CaP was not verified.

Alexandros Besinis et. al. recently examined silica and chemical synthesized HAp nanoparticles as potential candidates for tissue regeneration strategy [40, 41]. Fully demineralized dentin substrates were immersed in solution containing silica nanoparticles as well as chemically synthesized HAp nanoparticles. This resulted in infiltration of the dentin bars with respective nanoparticles. Silica nanoparticles showed higher remineralizing capacity than chemically
synthesized HAp nanoparticles after exposure to the artificial saliva solution. Loss of HAp particles was also seen after a week of immersion period whereas silica nanoparticles showed greater capacity to adhere to the collagen matrix as well as attract Ca and P minerals. The limited remineralization potential due to loss of nanoparticles suggested the inability of the HAp to bind to the collagen fibrils of demineralized dentin.

The drawbacks of these previously tried techniques can be insufficient adhesion of infiltrated nanoparticles to the dentin substrate. Moreover, complete remineralization of the demineralized dentin was not achieved. Trends also showed resulting demineralization over a period of time. Thus, it is required to come up with a technique which can lead to infiltration of particles which can chemically attract mineral ions as well as can adhere to the substrate even after exposure to remineralizing solution.

1.4 Atomic Layer Deposition on tooth HAp

Atomic layer deposition is a potential technique for remineralization of dentin which is supposed to have ability to overcome drawbacks of all the previous techniques. Being a pinhole free and conformal deposition technique, [42] ALD has the potential to coat entire exposed area of dentin.

1.4.1 Infiltration of the dentin with metal oxide

The present study deals with a high aspect ratio structure. Dentin is a porous structure which can be described with the help of dimensionless aspect ratio. Aspect ratio is a characteristic of the geometry [12]. In the case of a cylindrical pore which is open at the ends, aspect ratio can be defined as length of the pore divided by the pore diameter i.e. length of the major axis divided
by width of the minor axis [43]. In the case of dentin with average diameter of around 1.2 μm with 1 mm thick section, the aspect ratio is calculated to be approximately equal to 834. Thus, dentin block of 1 mm thickness can be regarded as a high aspect ratio structure.

1.4.2 Research findings and challenges

1.4.2.1 Effect of low pressure

Drying of tooth matrix in vacuum has been reported in literature. Applying vacuum results in drying of tooth matrix with loss of water of constitution or crystallization. The chemical structure of hydroxyapatite is conserved with modifications in the physical appearance of the tooth matrix due to loss of water of crystallization. It has been reported that at low pressure condition (10^{-3} \text{ bar}) the water molecules are lost by the Ca hydroxyapatite crystals [44]. The effect of low pressure i.e. vacuum needs to be verified before ALD experiment.

1.4.2.2 Effect of elevated temperature on tooth structure

Structural and chemical changes occurring in tooth have been reported in literature. It is observed that the tooth matrix is not stable above certain temperature and shows structural changes [45]. The changes are reported for wide range of temperatures. It is reported that the thermal treatment up to 300 °C does not result in any visible changes of the enamel color and it cannot be determined visually. Visual changes such as discoloration and blackening are observed above 400 °C. These changes are directly related to the level of carbonization and incineration of teeth [46]. This can be attributed to thermal decomposition of hydroxyapatite crystals. It is also reported that irreversible damage to dental pulp (in vivo conditions) starts to occur when temperature reaches a
critical value which is 42.5 °C [47]. Thermal properties of enamel and dentin are listed in the Table 1.1.

Table 1.1: Thermal properties of Enamel and Dentin[48]

<table>
<thead>
<tr>
<th>Property</th>
<th>Enamel</th>
<th>Dentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastic modulus (GPa)</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>Thermal conductivity [*10⁻⁴ J/(mm s °C)]</td>
<td>9.37</td>
<td>5.84</td>
</tr>
<tr>
<td>Coefficient of thermal expansion (*10⁻⁶/°C)</td>
<td>11.4</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Due to difference in thermal expansion coefficient of enamel and dentin, thermal stresses are responsible for cracking of tooth components. Thus, cracks are also observed at elevated temperatures along with discoloration.

1.5 Characterization Techniques

1.5.1. Degassing experiment

ALD reactor is a complex system which works under vacuum. The substrate (specifically of biological origin) undergoing ALD reaction can contain substances that evaporate under vacuum resulting in outgassing from the substrate. This can lead to entrapment of air bubbles in the vacuum system resulting in disturbance in working of the complex ALD system. Thus, it is necessary to check the compatibility of the substrate with vacuum for ALD at the desired temperature. Thus, a vacuum chamber is designed in the ‘Advanced Materials Research Laboratory’ to verify the compatibility of biological samples under vacuum. As shown in Figure 1.8, the vacuum chamber can be connected to the heating coil and has in built pressure gauge connected to continuously monitor changes in the pressure inside the chamber.
Initially the vacuum is turned on without putting any sample inside the chamber. After the pressure reaches to minimum possible value, the vacuum is turned off and the time it takes to reach the atmospheric pressure is recorded. Similar experiment is repeated by putting the substrate inside the chamber and time is recorded again. If the substrate experiences any outgassing, the vacuum chamber will consequently take less amount of time to reach to the atmospheric pressure. Hence, the difference in the recorded time in both the cases is the measure of outgassing by the substrate. Weight measurements are also taken before and after the degassing experiment to validate the observation. The substrate is compatible with the vacuum if no significant difference in the recorded time as well as weight measurements is obtained. The heating system allows to run the experiment at desired temperature.
1.5.2. *Scanning Electron Microscopy*[49]

The scanning electron microscopy is a surface imaging tool which is useful in analyzing surface morphology of the substrate by scanning if with a beam of focused electrons. It generally operates in 2 modes namely ‘Secondary Electron Mode’ and ‘Back-scattered Electron Mode’. Secondary electron mode is based on the detection of secondary electrons that are emitted from the sample as a result of inelastic scattering of the surface atoms by collision with beam electrons. This collision leads to the transfer of energy from the electrons to surface atoms resulting in emission of secondary electrons from the samples. Back-scattered electrons are the high-energy electrons that are reflected from the surface of the sample as a result of elastic collision with the surface atoms. SEM images are produced by the detection of these electrons (depending on the mode of operation of SEM) with respect to the position of the beam on the sample. For the purpose of this study, back-scattered mode of SEM is mainly used.

1.5.3. *Energy Dispersive X-ray Spectroscopy*

Energy dispersive x-ray spectroscopy (EDS) is a chemical analysis tool to detect elemental composition of the material. It works on the basic principle that every element has a unique atomic structure resulting in unique X-ray emission spectra. The sample to be analyze is bombarded with a focused beam of charged particles (such as electrons or protons) or X-rays. The incident beam can excite the electron in inner shell of the atom, ejecting it from the shell. This results in creating a hole in that inner shell which can be filled up by an electron in the outermost shell. The difference in the energy between higher-energy and lower-energy shell is sometimes released in the form of X-rays. The energy and number of X-rays emitted from the sample provide information about the elemental composition of the sample.
1.6. Summary and Objectives

Atomic layer deposition continues to gain interest due to vast variety of applications including biomaterials. Moreover, infiltration and remineralizing potential of various nanoparticles and materials is continued to be investigated in order to find out a definite solution to dental decay. Tremendous efforts are being taken in order to search for a curative technique for dental demineralization. Atomic layer deposition technique can be an emerging tool having ability to overcome disadvantages of previous techniques of tissue regeneration strategy. Owing to the porous structure of dentin component of tooth and ability of ALD to coat complex structures with uniformity, a pinhole free and chemically deposited layer of metal oxide can is expected. This study is aimed to accomplish two objectives, the first, to examine the potential of HAp crystals for surface nucleation and adsorption of precursors in order to enable nano coating by atomic layer deposition and the second, to evaluate and characterize the ability of as-deposited titanium dioxide film to allow nucleation of calcium phosphate for biomimetic remineralization study. Furthermore, the next step research in order to improve the results of present study as well as direct ALD deposition of CaP is also proposed.
Chapter 2

Atomic Layer Deposition:

A novel strategy to restore calcium hydroxyapatite of tooth

Abstract

We demonstrated that the calcium hydroxyapatite matrix of dentin can be infiltrated with a nanofilm of TiO$_2$ by low temperature atomic layer deposition. Dentin is a high aspect ratio structure having dentinal tubules throughout its depth with pore openings of 1.21 µm diameter on the top surface. Atomic layer deposition (ALD) was performed on teeth samples at 100 °C using tetrakis(diethylamino)titanium as a precursor and ozone as an oxidizer to obtain a 42 nm-thick layer of TiO$_2$. Energy dispersive x-ray spectroscopy (EDS) showed that a nanofilm of TiO$_2$ was deposited on the top surface of the dentin as well as the exposed surface of dentinal tubules throughout their depth. We further confirmed the potential of as-deposited TiO$_2$ nanofilm to nucleate calcium phosphate crystals and remineralize the dentinal matrix. Calcium and phosphorus content of the demineralized dentin was found to increase by a factor of 5 due to nucleation and crystal growth as a result of in vitro biomimetic remineralization process. Atomic layer deposition of calcium phosphate nucleating metal oxide is a promising technique to restore the lost minerals from teeth.
2.1 Introduction

Dental caries, caused by endogenous bacteria in the biofilm (Streptococcus mutans and Streptococcus sobrinus) is the most prevailing and chronic disease affecting people at different ages worldwide [32]. The organic acids produced by these bacteria present on the biofilm adhering to the tooth surface lower down the salivary pH value (less than 5) [50]. Increased concentration of H⁺ ions results in lower concentration of OH⁻ ions as the [H⁺] *[OH⁻] product is always constant at any given temperature. The phosphate group mainly exists in protonated form (H₃PO₄, H₂PO₄⁻ & HPO₄²⁻) at pH lower than 5 and PO₄³⁻ form is present in very small amount. This results in dissolution of OH⁻ and PO₄³⁻ ions from the hydroxyapatite (HAp) matrix of tooth into the saliva solution which is undersaturated with respect to those ions at low pH values [50]. This demineralization of tooth is counterbalanced by deposition of minerals from saliva solution. Dental caries results when the balance between simultaneous demineralization and remineralization is not maintained. Epidemiology of dental caries shows that along with increased lifespan of teeth and due to advanced dental techniques and tools, dental decay has become more frequent in aged adults which demands expensive treatments [32]. Due to expensive treatments, 90% of the decay cases are left untreated [31]. During the early stages of caries the collagen structure is left intact in spite of the loss of minerals [32] thus, making the dental hard tissue remineralization strategy possible.

Human tooth consists of 3 hard tissue components viz. enamel, dentin and cementum. 80% of the dentin, a hard tissue component of tooth, is made up of calcium hydroxyapatite [Ca₅(PO₄)₃(OH)] [25, 29]. Its hardness is 3-4 on the Mohs scale. Dentin is bone-like matrix characterized by multiple closely packed dentinal tubules that traverse its entire thickness [51]. Mean diameter of dentin tubule is about 1.21 ± 0.08 µm and the distance between adjacent tubules
is 5-10 μm [29]. Dentinal tubules are pores which are present longitudinally throughout the dentinal hydroxyapatite matrix with length of about 2 mm.

Tissue regeneration strategy is to infiltrate this dentin matrix with minerals or mineral attracting chemical species. Various techniques have been tried to restore the lost minerals into the HAp matrix. In vitro studies were performed to analyze remineralization potential of bioactive glass S53P4 (53 mol% SiO₂, 23 mol% Na₂O, 20 mol% CaO and 4 mol% P₂O₅) to observe that the bioactive glass decreases the degree of decalcification [37]. Another study performed with ultrafine bioactive glass particles resulted in infiltration within the HAp matrix with mechanical stability well below that of natural dentin [38]. The lack of mechanical reconstitution was attributed to imperfect arrangement of newly deposited mineral. Alexandros Besinis et. al. tried infiltration of dentin with silica nanoparticles and chemically synthesized HAp nanoparticles by immersing them in the solution containing those nanoparticles [40, 41]. The result proved successful infiltration with nanoparticles but failed to retain the minerals after exposure to artificial saliva solution. The negative result can be attributed to insufficient adhesion of nanoparticles to the HAp matrix and thus their diffusion from the surface after exposure to biomimetic saliva solution. Difficulties encountered with all the prevailing and developing techniques were non-uniform infiltration, insufficient adhesion to dentin hard tissue, removal during in vitro studies and inability to nucleate calcium phosphate in biomimetic coating process.

In the present study, we use atomic layer deposition (ALD) technique to coat the dentin tubules with TiO₂ due to its ability to form highly conformal films. Being deposited by a chemical deposition process, the highly conformal thin film will be adhered to the surface in such a way that after exposure to the artificial saliva solution, it will not be removed from the surface. Titanium
oxides are commonly used in dental and bone implants as well. TiO$_2$ has been tried for coating on various biomaterials owing to its biocompatibility, mechanical strength and comparatively reasonable cost [17-19]. ALD driven infiltration of biomaterials with metal oxide particles has recently been tried on few substances such as spider dragline silk, collagen membrane and eggshell collagen membrane to analyze characteristics of the process and effect of as-deposited metal oxide on mechanical strength of the biomaterials [17-19]. ALD on these substances was successful due to the chemical structure of the surface of those substrates. In case of dentin, the chemical structure at the surface of hydroxyapatite matrix may allow nucleation of the precursor.

The atomic layer deposition will be able to infiltrate dentin matrix with TiO$_2$ due to its ability to form highly conformal films with sufficient amount and exposure time of precursor molecule. HAp has \([-\text{OH}]\) and \([-\text{P=O}^-]\) groups on the surface [52]; hence the exposed top and tubular surface can be considered as potential nucleating site for desired precursor molecule. Due to high aspect ratio of the sample (~1000) and high surface area; precursor depletion could be encountered owing to rapid precursor consumption [12]. It is known that HAp changes its chemical structure when exposed to high temperature (more than 200 °C). Also, carbonization and blackening along with cracking of the structure is observed when the temperature goes beyond 400 °C [44, 45, 48]. Mechanical cracking of the tooth HAp can be attributed to difference in thermal expansion coefficients of enamel and dentin [48]. Low temperature ALD process is chosen for the present study to avoid chemical and mechanical changes occurring at higher temperatures.

Moreover, TiO$_2$ has potential to attract minerals from the solution and facilitate the remineralization process [53]. The potential of SiO$_2$ to accelerate heterogeneous nucleation of calcium phosphate crystals has been investigated previously [40]. Heterogeneous nucleation of
CaP is considered to be initiated by adsorption of Ca\(^{+2}\) ions on negatively charged surface.[54] Isoelectric point of TiO\(_2\) being around 6, surface carries net negative charge at pH>6 (physiological pH) in aqueous solutions [55, 56]. High Lewis base surface tension parameter is responsible for CaP growth on the TiO\(_2\) coated surface [57]. Morphology and chemical properties have cooperative influence on surface precipitation reactions.

In summary, vapor deposition studies on hydroxyapatite matrix of human dentin have not been performed yet. Being a porous structure it is required to study if metal oxide particles can infiltrate the structure by vapor deposition technique, ALD in this case. Possibilities could be pore blockage due to deposition of TiO\(_2\) nanofilm along with infiltration as well as insufficient nano-coating on the tubular surface. SEM studies are performed on the top surface as well as cross section of the dentin. Cross sectional SEM-EDS analysis is performed to investigate the infiltration depth. The remineralization capability of TiO\(_2\) is studied by keeping the dentin samples in artificial saliva solution and SEM-EDS analysis is used to investigate changes in mineral content.

2.2 Materials and Methods

2.2.1 Dentin sample preparation

Blocks of dentin were obtained from previously extracted human molar teeth. Approval for using human teeth has been obtained from Institutional Review Board of University of Illinois at Chicago, USA. (IRB#: 2014-1065) Specimens of 1±0.2mm thickness were obtained from mid-coronal dentin and cut with a slow-speed diamond saw under constant water irrigation according to the previously reported procedure [58]. The prepared dentin samples were preserved in 10% w/v sodium azide (NaN\(_3\)) solution (aq) to prevent any microbial growth.
2.2.2 Acid etching and drying of the sample

Acid etching was performed on all the cut dentin samples to remove the smear layer present on hydroxyapatite matrix and thereby to expose the dentin tubules. 32% w/w phosphoric acid gel was applied for 20 seconds [59] on the entire surface of the sample followed by rinsing with deionized water for 20 seconds. The acid etched samples were kept for air-drying at room temperature (21°C) for 24 hours to remove excess moisture.

2.2.3 Before ALD: Degassing experiment

Degassing experiment was performed on a batch of 3 samples to study their compatibility under vacuum at room temperature (22°C) as well as at higher temperatures (150 °C and 250 °C). 10⁻³ Torr vacuum was used for the same purpose. (Table 2.1)
Table no. 2.1: Summary of the degassing experiment runs and experimental conditions (temperature vacuum and time conditions)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Vacuum (10⁻³ Torr)</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>R.T. (22 °C)</td>
<td>15 minutes</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>150 °C</td>
<td>15 minutes</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>150 °C</td>
<td>60 minutes</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>250 °C</td>
<td>15 minutes</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>250 °C</td>
<td>60 minutes</td>
</tr>
</tbody>
</table>

The pressure was allowed to drop down by turning the vacuum on. As the pressure reaches minimum possible value (10⁻³ Torr), the vacuum was turned off to calculate the time required to again reach atmospheric pressure. The time taken by chamber with and without the sample (empty chamber) was compared to check the vacuum compatibility of dentin samples. The methods to evaluate the degassing experiment were visual, difference in weight measurement and the pressure difference obtained by degassing.
2.2.4 Atomic Layer Deposition

![Diagram of Atomic Layer Deposition process]

**Figure 2.1: Schematic diagram of the Atomic Layer Deposition process (TiO$_2$ deposition)**

After checking the compatibility of tooth samples with vacuum and high temperatures, the new samples were taken for infiltration process using Atomic later deposition (ALD) reactor (fig. 2.1). As the maximum tolerated temperature was found to be 150 °C, low temperature ALD was performed on the hydroxyapatite matrix to deposit TiO$_2$ thin film. The ALD/CVD hybrid system built by Selvaraj et. al., explained in previous paper, was used for performing deposition of TiO$_2$ [60]. The ALD system was first calibrated and the air-dried samples were kept in deposition chamber along with an evidence (silicon) sample. TDEAT i.e. tetrakis(diethylamino)titanium was used as a precursor and Ozone as an oxidizer. Ozone generator was used for producing ozone of
1000 ppm concentration. The reactor temperature was kept at 100 °C (substrate temperature) and the operating pressure at 0.5 torr. The TDEAT (precursor) bubbler temperature was kept constant at 65 °C. Precursor and oxidizer pulse duration were set as 16s and 1s respectively and precursor and oxidizer purge times were decided as 25 s each. 300 cycles were run for the infiltration to occur. The TiO\textsubscript{2} film thickness was measured with spectroscopic ellipsometry (J.A. Woollam Co. M-44) with the help of an evidence sample.

2.2.5 Scanning Electron Microscopy(SEM)- Energy Dispersive Spectroscopy(EDS) analysis

SEM (Hitachi S-3000N, variable pressure SEM) was used to observe the top surface as well as cross section of the experimental and control samples. Thickness of the sample for top layer examination was 1 mm and that for cross section was 2 mm. SEM was performed at the accelerating voltage of 5 kV. EDS examination was performed using the SEM device equipped with an EDS detector. The dentin hydroxyapatite samples with a TiO\textsubscript{2} nanofilm of approximately 45 nm thickness were probed with an Energy Dispersive X-ray Spectrophotometer (Oxford Inca EDS system with a light element X-ray detector, 15mm WD). Identical operating conditions of beam energy and scanning parameters were used for all EDS scans. Top surface and cross section was analyzed to confirm presence of TiO\textsubscript{2}. The voltage was set at 10 kV for preliminary ALD study and 5 kV for remineralization evaluation. The scan duration was set at 60 s for all the samples.

2.2.6 Demineralization of samples and pre-remineralization treatment

In order to study the remineralization potential of the TiO\textsubscript{2}, new dentin samples were first demineralized using 10% phosphoric acid for 2.5 hours. Temperature and vacuum compatibility of the demineralized samples was checked using degassing experiment. The demineralized
samples were then rinsed with distilled water to remove residual acid content and air-dried at 22 °C for 24 hours. The demineralized samples were grouped into 2 categories viz. control samples and test samples. ALD was performed on the test samples by the procedure explained previously in section 2.2.4.

2.2.7 Remineralization using infiltration solution:

The test and control samples were immersed in artificial saliva i.e. remineralizing solution. The grouping of samples was done as shown in Table 2.2.

Table no. 2.2: Summary of sample groups for biomimetic remineralization study with conditions: Temperature - 37oC, pH > 6.5 and minimal stirring speed. Control sample: dentinal block of 1 mm thickness air dried for 24 hours; Test samples: as-deposited dentinal block of 1 mm with atomic layer deposition of TiO2

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 0 Study (no immersion in artificial saliva solution)</th>
<th>Control sample (dentin with no TiO2 deposition)</th>
<th>Test sample (dentin with as-deposited TiO2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2</td>
<td>Week 1 study (immersion in artificial saliva solution for a period of 1 week)</td>
<td>Control sample (dentin with no TiO2 deposition)</td>
<td>Test sample (dentin with as-deposited TiO2)</td>
</tr>
<tr>
<td>Group 3</td>
<td>Week 2 study (immersion in artificial saliva solution for a period of 2 week)</td>
<td>Control sample (dentin with no TiO2 deposition)</td>
<td>Test sample (dentin with as-deposited TiO2)</td>
</tr>
<tr>
<td>Group 4</td>
<td>Week 3 study (immersion in artificial saliva solution for a period of 3 week)</td>
<td>Control sample (dentin with no TiO2 deposition)</td>
<td>Test sample (dentin with as-deposited TiO2)</td>
</tr>
</tbody>
</table>
The components and their concentration in the remineralizing solution is listed in the Table 2.3 [61]. The solution was prepared using distilled-deionized water and the pH was adjusted to 6.5 using HEPES buffer. The samples were immersed in the remineralizing solution for the desired duration in conical centrifuge tubes at 90 RPM and 37 °C to mimic physiological condition. The pH of the solution was checked periodically and maintained at 6.5. The solution was replaced every week to avoid effect of pH change.

Table no. 2.3: Chemical composition in artificial saliva (remineralization) solution

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KCl</td>
<td>0.8g/L</td>
</tr>
<tr>
<td>2</td>
<td>NaCl</td>
<td>0.4g/L</td>
</tr>
<tr>
<td>3</td>
<td>CaCl₂·2H₂O</td>
<td>0.362g/L</td>
</tr>
<tr>
<td>4</td>
<td>NaH₂PO₄·2H₂O</td>
<td>1.38g/L</td>
</tr>
<tr>
<td>5</td>
<td>Na₂S·9H₂O</td>
<td>0.005g/L</td>
</tr>
<tr>
<td>6</td>
<td>NaN₃</td>
<td>0.2g/L</td>
</tr>
<tr>
<td>7</td>
<td>HEPES buffer</td>
<td>20 mmol</td>
</tr>
</tbody>
</table>

The samples were removed from the artificial saliva solution at every interval and analyzed for the mineral content with SEM-EDS. Top section and cross section of the sample were analyzed at every stage to investigate the probable remineralization.
2.3 Results and Discussion

2.3.1 Degassing of tooth Calcium hydroxyapatite matrix

Time required by the vacuum chamber with and without the dentin sample to reach the atmospheric pressure was noted down at different temperatures (R.T. 22 °C, 150 °C and 250 °C) after turning the vacuum off. No significant time difference (in seconds) was observed suggesting no outgassing from the sample. The empty vacuum chamber took approximately 4 minutes to reach from 1 mili Torr to 0.5 atm; whereas the same vacuum chamber containing a dentin sample took 5-10 seconds less than 4 minutes to reach to the 0.5 atm every time. The samples were kept in the degassing chamber for a period of 15 minutes. Weight measurements were taken before and after the degassing experiment. In run 1 i.e. in the vacuum at room temperature, no significant weight difference was seen (>1%) but at higher temperatures (150 °C and 250 °C) 5% weight reduction was observed which can be attributed to the loss or evaporation of ‘water of crystallization’ that was originally present in tooth hydroxyapatite. The dentin component of human tooth contains 10% water and enamel contains 2% water which is absorbed on the mineral surface or between the crystals. Also, the HAp molecule \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]\) is expected to lose OH group when heated to higher temperature [44]. Presence of these water molecules explains the weight loss at 150 °C and 250 °C and no significant weight difference at room temperature which is below boiling point of water. Heat treatment of 1 hour changed the appearance of sample at 250 °C whereas change was observed at 150 °C. At elevated temperatures, increased level of carbonization is responsible for brownish discoloration of calcium hydroxyapatite. The teeth samples were found to be compatible with the vacuum (10^{-3} Torr) at room temperature as well as
at 150 °C. Temperature of 100 °C was set for all the next experiments to avoid change in composition and thereby appearance.

2.3.2 Infiltration of Ca hydroxyapatite matrix of dentin with TiO$_2$ using ALD

EDS analysis confirmed the presence of TiO$_2$ on the exposed surface of HAp matrix. HAp matrix of tooth is made up of a central Ca(OH)$_2$ and three surrounding Ca$_3$(PO$_4$)$_2$ groups [62, 63] due to which surface of the exposed part of HAp contains oxygen ions. These oxygen ions provide nucleating sites for the TDEAT precursor followed by further oxidation with ozone leading to TiO$_2$ monolayer.
Figure 2.2: SEM images showing Morphology (A) SEM image of Top surface of the dentin sample without ALD treatment (control sample); dentinal block of 1 mm thickness acid etched with 32% w/w phosphoric acid gel for 20 seconds and air dried for 24 hours at 22 °C (room temperature conditions) (B): SEM image of Top surface of the as-deposited dentin sample with 42 nm thick TiO$_2$ layer (test sample). TDEAT is used as a precursor with ozone as an oxidizer for a pulse duration of 16 s and 1 s respectively. N$_2$ purge time is set as 25 s after precursor and oxidizer pulse. Operating temperature is set as 0.5 Torr and 100 °C as deposition temperature for a total 300 cycles.
Both HAp (Fig. 2.2(A)) and HAp/TiO$_2$ (Fig. 2.2(B)) surfaces show unchanged morphological features which can be attributed to conformal coating by atomic layer deposition. TiO$_2$ film of approximately 42 nm was obtained on the top surface with previously specified system parameters. Conformal coating without any cracking of HAp is observed. Fig. 2.3 represents EDS analysis plot at one point on the as-deposited sample clearly showing presence of Ti on the top surface of HAp of dentin sample.
Figure 2.4: (A): Cross sectional SEM image of dentin sample with TiO$_2$ coated tubules by atomic layer deposition (test sample). Experimental conditions are as described in section 2.2.4 (B): EDS confirms the presence of Titanium, oxygen, calcium and phosphorus in the cross section of the dentin (in tubules) (C): Distribution of the elements in the cross section of the dentin sample with as-deposited TiO$_2$ shown by EDS analysis based on the signals detected.

Fig. 2.4A represents the cross section of the as-deposited dentin sample where 1 mm long distinct tubules were observed. The tubular channels were probed with EDS for the presence of titanium at different depths from the top surface. Fig. 2.4B shows presence of Ti on the dentinal tubule. Mapping of the elements (Ti, O, Ca and P) was also done using the same EDS analysis tool (Fig. 4C) to see the distribution of elements in the cross section of dentin samples. Mapping of the
elements is based on the signals received from the site. (Table 2.4) The average Ti atom% observed in 3 different tubules at different places was 26.6 ± 2.6%. Also, 5 points were probed at different depths from top to bottom on the dentinal tubule to analyze the depth profile which gave 27.3±0.83% Ti atom% showing uniformity of titanium content throughout a single tubule channel. It was observed that standard deviation for inter-tubular Ti atom% values is higher than that for Ti atom% values of the same tubule by around 2%. This can be attributed to difference in exposed surface area and diameter (pore opening) of the different tubules. The Ti atom% values (Table 2.4) suggest uniform coating throughout the exposed area of dentin tubule. Based on these results, it was proved that the long exposure ALD avoided the precursor depletion due to high aspect ratio of tubules and TiO₂ was seen throughout the hydroxyapatite matrix at low temperature (100 °C).

Table no. 2.4: Ti atom% determined by SEM-EDS at different depths from top layer in a cross sectioned dentin block showing dentinal tubules having nano-coating of TiO₂ by atomic layer deposition. The Ti atom% values are for one dentinal tubule

<table>
<thead>
<tr>
<th>Distance from top surface (approximate)</th>
<th>Ti atom%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mm</td>
<td>26.8</td>
</tr>
<tr>
<td>0.3 mm</td>
<td>27.7</td>
</tr>
<tr>
<td>0.5 mm</td>
<td>26.5</td>
</tr>
<tr>
<td>0.8 mm</td>
<td>28.3</td>
</tr>
</tbody>
</table>
2.3.3 Remineralization potential of dentin block infiltrated with TiO$_2$

Top surface and cross sectional surface of week 0, week 1, week 2 and week 3 samples were analyzed for possible Calcium phosphate nucleation and eventually, remineralization. Morphological changes are observed using SEM imaging and chemical changes are analyzed using EDS technique.
Figure 2.5: (A): SEM image of the top surface of dentin sample (control sample in remineralization study) demineralized by immersing in 10% w/w phosphoric acid solution for 2.5 hours and kept in remineralization solution for a period of 1 week; Conditions: 37 °C, pH> 6.5 and minimal stirring speed (B): SEM image of top surface of as-deposited dentin sample (ALD conditions as described in section 2.4) kept in remineralization solution for a period of 1 week showing no appreciable accumulation of nucleated Calcium Phosphate on the top surface (C): SEM image of the top surface of dentin sample (control sample in remineralization study) demineralized by immersing in 10% w/w phosphoric acid solution for 2.5 hours and kept in remineralization solution for a period of 2 weeks; Conditions: 37 °C, pH> 6.5 and minimal stirring speed (D): SEM image of top surface of as-deposited dentin sample (ALD conditions as described in section 2.4) kept in remineralization solution for a period of 2 weeks showing accumulation of nucleated Calcium Phosphate on the top surface (E): SEM image of the top surface of dentin sample (control sample in remineralization study) demineralized by immersing in 10% w/w phosphoric acid solution for 2.5 hours and kept in remineralization solution for a period of 3 weeks; Conditions: 37 °C, pH> 6.5 and minimal stirring speed (F): SEM image of top surface of as-deposited dentin sample (ALD conditions as described in section 2.4) kept in remineralization solution for a period of 3 weeks showing accumulation of nucleated Calcium Phosphate on the top surface
The figures (Fig. 2.5(A)-2.5(B)) represent the SEM images of top surface of the dentin samples kept in the biomimetic remineralization solution for a period of 1 week. No morphological difference or nucleation of Calcium phosphate crystals was seen on the top surface of control as well as test samples. The figures (Fig. 2.5(C)-2.5(D)) represent the SEM images of top surface of dentin samples demineralized using 10% w/w phosphoric acid solution followed by the treatment with artificial saliva solution for a period of 2 weeks. And the figures (Fig. 2.5(E)-2.5(F)) represent the similar group of week 3 samples. In case of week 2 and 3 samples, different topography was observed with SEM imaging showing clusters of calcium phosphate due to nucleation as a result of biomimetic remineralization process (Fig. 2.5(C)-2.5(F)). No such surface nucleation was seen on the top surface of control samples pretreated in a similar manner followed by 3 week remineralization process (Fig. 2.5(C), 2.5(E)). Both week 2 and week 3 group of test samples (dentin with as deposited TiO₂) showed nucleation of calcium and phosphorus but in case of week 3 samples more nucleated crystals were observed and larger top surface area was observed to be covered with calcium phosphate crystals. This also suggests that the nucleation was accelerated by pre-nucleated calcium and phosphorus. Different points on the top surface (Fig. 2.6(A)) such as area between pores, on the opening and area with different morphological features such as small as well as larger crystal clusters were probed for Ca and P elemental atom%. From the plot obtained from EDS analysis data (Fig. 2.6A-2.6B) obtained at 3 different points from top surface of 3 week remineralized as-deposited dentin sample, it can be seen that Ca and P atom% is not uniform all over the surface as shown in Fig. 2.6B. Few sites with comparatively smaller clusters showed less Ca and P content suggesting initiation of nucleation phenomenon. Moreover, sites with larger clusters (approximately 0.1 mm in diameter) showed highest Ca and P content. These larger
clusters block many tubular openings i.e. dentinal pores as the average diameter of pores is around 1.2 μm. Ti content from that site was not detected suggesting remarkable cluster height blocking Ti signals. The titanium content was uniform on the top surface confirming conformal TiO$_2$ nanolayer. Drastic difference between the topography of control and as-deposited samples from week 3 was observed unlike week 1.

![SEM image of top surface of as-deposited dentin sample with 3 week remineralization study showing clusters of nucleated calcium phosphate crystals. Point no. 1 corresponds to area on the top surface between the dentinal pore openings; point no. 2 corresponds to smaller Calcium phosphate crystals and point no. 3 corresponds to larger nucleated Calcium phosphate crystals as a result of biomimetic remineralization process. Graphical analysis of EDS data at these points is given in figure no. 2.4; (B): Comparison of elemental atom% by SEM-EDS analysis (with respect to Ca and P content) between different points on top-surface of as-deposited sample (shown in figure 5(A)) which was kept in biomimetic remineralization solution for a period of 3 weeks; Conditions: 37 °C, pH > 6.5 and minimal stirring speed]

Along with the remarkable morphological difference, mineral content profile was studied for a period of 3 weeks (Fig. 2.7-2.8). The dentin is composed of hydroxyapatite crystals embedded in a collagen matrix which is made up of collagen type I and other phosphoproteins. As all the
dentin samples are demineralized prior to the ALD and remineralization process, Ca and P content drops to the minimum value with high amount of C content due to presence of collagen matrix. P content is found higher due to presence of phosphoproteins in the organic content of dentin. Figure 7 and 8 show very low Ca and P atom% values around 1.03% and 5.28% respectively due to demineralization process. Ca atom% data shows that the Ca content in control samples (without ALD treatment) goes down throughout the experimental period of 3 weeks whereas it shows increase in case of as-deposited samples (Fig. 2.7). Due to demineralization there are less mineral ions present in the matrix to hold Ca$^{+2}$ by electrostatic force of attraction due to which when exposed to the biomimetic saliva solution, dentin tends to lose Ca$^{+2}$ ions which also suggests that the rate of loss of minerals is faster than that of remineralization in case of control sample resulting in decrease in the Ca atom% content leading to loss of Ca$^{+2}$ in control samples. P atom% profile of control samples shows similar behavior over a period of 3 weeks. (Fig. 2.8) Calcium atom% content increases by a factor of 2 in a period of one week in case of as-deposited samples. Initial concentration of Ca is not remarkable implying not much increment in the mineral content. Remarkable increase (by a factor of 5) in Ca atom% is observed during the time period of 2 weeks. P atom% in case of as-deposited samples shows different behavior. P atom% content shows decrease at the end of 1st week and remarkable increase at the end of 3rd week. In case of week 3 samples, the Ca atom% and P atom% on the top surface of the larger nucleated crystals is 24.0% and 20.5% respectively, showing appreciable nucleation at few sites. Difference between week 0, 1, 2 and 3 control samples with respect to mineral content represents remineralization process in the absence of TiO$_2$ nanofilm and that between as-deposited samples represents the effects of TiO$_2$ nanofilm.
In summary, week 2 and week 3 SEM analysis of the top surface of dentin samples showed similar morphological features suggesting that the nucleation takes place mainly after a period of 1 week as no such remarkable crystal growth was observed after week 1.

![Graph showing Ca atom% vs. remineralization period for control and as-deposited dentin samples](image)

**Figure 2.7:** Plot of Ca atom% vs. remineralization period for control and as-deposited dentin samples to assess biomimetic remineralization process. The elemental atom% values of Ca are calculated on the top surface of the sample. Values at 3 different locations are measured to account for variability in the remineralization process.
Figure 2.8: Plot of P atom% vs. remineralization period for control and as-deposited dentin samples to assess biomimetic remineralization process. The elemental atom% values of P are calculated on the top surface of the sample. Values at 3 different locations are measured to account for variability in the remineralization process.

High standard deviation for Ca and P atom% values of as-deposited samples which are exposed to biomimetic remineralization process for a period of 2 and 3 weeks can be attributed to different morphology at different points due to variation in crystal growth. Nucleation is favored at site with higher roughness due to presence of kinks. Apart from that, presence of pre-nucleated crystals accelerate further nucleation owing to similar chemical and physical structure of pre-nucleated and nucleating calcium phosphate.

The isoelectric point of TiO₂ is reported to be in between pH 5.8 – 6.2 suggesting net negative charge on the surface at salivary pH which is more than 6.5. In the aqueous saliva solution
TiO$_2$ is present in its hydrated form having -TiOH and -TiO$^-$ groups on the surface [64] accelerating adsorption of Ca$^{+2}$ ions possible. The Na$^+$ ions from the remineralizing solution are responsible for limited adsorption (absolute value) of Ca$^{+2}$ ions. Adsorption of phosphate groups requires positively charged surface for electrostatic attraction thus Ca$^{+2}$ adsorption could be considered as a prerequisite for adsorption of phosphate groups and eventually crystal growth of hydroxyapatite. Lewis acidic sites of Ti$^{+4}$ are not seen to be functional in case of pH greater than its isoelectric point making adsorption of (PO$_4$)$^{3-}$ difficult.

TiO$_2$ nanofilm being negatively charged at physiological pH accelerated heterogeneous nucleation of calcium phosphate to restore the mineral matrix [53]. Pre-nucleated calcium phosphate clusters further enhance the surface nucleation process due to presence of initial nucleated crystals leading to crystal growth. It can be expected that the surface will be covered with calcium phosphate layer when exposed to remineralizing solution for more amount of time.

Cross section of the dentin samples was also observed to examine difference in mineral content profile and morphological changes between control as well as test (as-deposited) samples for possible remineralization. Atom% values of Ca and P obtained from EDS data at different depths of all the samples were appreciably high for samples from all the groups. It was noticed that the HAp matrix was not demineralized sufficiently after 0.2 mm depth from the top surface because of the non-uniform and insufficient demineralization process i.e. by immersing in phosphoric acid of optimized concentration. These results can be attributed to the diffusion limited regime causing barrier to diffusion of phosphoric acid ions and inhibiting dissolution process of mineral ions into the demineralizing solution. Diffusivity of acid solution into the dentin tubules
is affected by different factors resulting in limited demineralization of the dentin block as follows [65]:

\[ D_{eff} = D_o \times \alpha \times \gamma \times \tau \]

Here, \( D_o \) is the diffusivity of acid ions without any diffusion barriers, \( \alpha \) is a factor that accounts for the effect due to increased viscosity near the solid surface, \( \gamma \) accounts for the electrostatic restrictions experienced by acid ions during diffusion into the dentin tubules, \( \tau \) is the tortuosity of dentinal tubules and \( D_{eff} \) is the effective diffusivity of the ions. The diffusivity \( D_o \) of acid ions under normal conditions and in the absence of diffusivity barriers is of the order of \( 10^{-5} \) cm\(^2\)/s. The diffusion length in the demineralization process is calculated based on the demineralization depth calculated from SEM-EDS analysis.

\[ \text{Diffusion Length} = 2 \times \sqrt{D_{eff} \times \text{time}} \]

Here, Diffusion length = 0.2 mm and time = 2.5 hours i.e. 9000s gives \( D_{eff} \) of the order of \( 10^{-8} \) cm\(^2\)/s. Thus, the impedance factor \( (f) \) which is equal to the product \( (\alpha \times \gamma \times \tau) \) is calculated to be of the order of \( 10^{-3} \) which is responsible for limited diffusion resulting in limited demineralization of the dentinal tubules.

Apart from that, no remarkable difference with respect to morphology as well as Ca and P atom\% values was observed between cross section of control and that of as-deposited samples after 1 week and 3 week immersion period unlike top surface. Diffusion coefficient of the mineral ions is lower in liquid media. Moreover, the porous HAp matrix of tooth and tortuosity of the tubules have high impact on effective diffusion coefficient of the mineral ions causing barrier to the diffusion process of ions similar to that in case of demineralization process. All these factors
are responsible for ineffective remineralization process in the region below top surface of the HAmp matrix.

The investigation clearly proved remineralization potential of TiO$_2$ nanofilm owing to the morphological difference observed between control and as-deposited samples. Chemical analysis of the nucleated crystals also confirm the presence of calcium phosphate crystals. Moreover, analysis of the HAmp matrix below the top layer showed limited remineralization potential of the TiO$_2$ nanofilm which is attributed to diffusion limited regime. However, limited demineralization prior to the in-vitro biomimetic remineralization process makes the argument more complex. Remineralization potential below the top layer of as-deposited dentin can be fully understood after successful demineralization process. Consequently, reducing diffusion barrier thus increasing effective diffusion coefficient can help understand remineralization potential of this novel technique below the top surface i.e. inside the HAmp matrix of dentin.

2.4 Conclusion

Dentin blocks of 1 mm thickness were found to be compatible with vacuum ($10^{-3}$ Torr pressure) and temperature up to 150 $^\circ$C. Moreover, low temperature atomic layer deposition of TiO$_2$ was successfully used to grow a 42 nm-thick film on the entire exposed surface of dentin which is a high aspect ratio structure using long exposure time of the precursor TDEAT. Uniform and conformal coating throughout the length of tubule (1 mm) was obtained with average tubular Ti atom$\%$ of 26.6 ± 2.6%. Consequently, apparent morphological difference was observed between the control and as-deposited samples after the biomimetic remineralization process with respect to nucleation and growth of calcium phosphate crystals. The TiO$_2$ nanofilm was found to be intact after 3 weeks of immersion in artificial saliva solution. Diffusion barriers for ion transport
through liquid media imposed limitations to the mineralization process at a great depth from the top surface. Our findings demonstrate the potential of novel strategy of coating dentin HAp with a nanofilm of TiO2 to successfully nucleate calcium phosphate on top surface of dentin because of likely electrostatic attraction between the nanofilm and mineral ions from remineralizing solution.
Chapter 3

Future Scope

3.1 Effective remineralization

In the present study, limitations were encountered due to diffusion processes. Firstly, the remineralization phenomenon was not effective due to insufficient diffusion of mineral ions as explained in section 2.3.3. Also, the ALD temperature is not suitable for in vivo studies. Following are the proposed modifications for future study.

3.1.1 Collapse of collagen fibers/structure of dentinal tubules

One possible reason behind insufficient remineralization of dentin substrate can be collapse of fibers. It is experimentally observed that proteinaceous substrates show collapse of protein network/collagen fibers after heating to higher temperatures [14]. It is also reported that acid treatment changes the hard mineral matrix to a soft demineralized collagen substrate that collapses after drying with air [66, 67]. Air-drying the dentin samples for 24 hours and exposing them to vacuum and high temperature conditions might have adverse effects on the original structure of collagen matrix. This can reduce the diameter of dentinal tubules and impose restrictions on the path of mineral ions during the remineralization period. To verify these changes, the remineralized samples (dentin with as-deposited TiO\textsubscript{2} nanofilm immersed in artificial saliva solution for a period of 3 weeks) were demineralized using 10% w/v phosphoric acid for 30 minutes to observe the changes in the structure of dentinal tubules in the cross section as well as top surface if any.
The collapse of collagen fibers/protein network after demineralization can lead to reduction in thickness of the sample due to severe acid conditions near the top surface. The thickness of the cross section of the sample was measured using SEM imaging. The original thickness of the dentin sample which was cut using slow speed diamond saw under continuous water irrigation was equal to 1 mm. The thickness of the experimental samples (demineralized samples which was originally demineralized with 10% \( \text{H}_3\text{PO}_4 \))
remineralized) was also equal to 1 mm. Thus, preliminary observation with respect to equal thickness of the sample after exposing to the acid conditions suggests intact collagen structure with no probable collapse of the network.

The cross section and top surface of these dentin samples were also observed and compared to the original demineralized samples. No remarkable morphological difference was detected in the top surface of the samples. Figure A shows top surface of the originally demineralized samples and Figure B shows top surface of the remineralized samples which are again demineralized using acidic conditions. Intact dentinal pores can be seen on the top surface in Figure B suggesting no distortion in the porous structure from top surface. Figures C and D show cross sectional images of the samples. Intact dentinal tubules are observed from the cross section of remineralized samples which are again demineralized prior to SEM imaging (Figure D). The SEM images of cross section (C and D) show no remarkable difference suggesting intact dentinal tubules even after exposure to acidic conditions/demineralization process.

Thus, from this demineralization experiment it can be concluded that there may not be any damage to the collagen network as no remarkable difference is detected from the SEM images. This is not a probable reason for inefficient remineralization.

3.1.2 Adjusting Viscosity of the remineralizing solution

Viscosity of the remineralizing solution can be a factor impeding diffusion of the remineralizing solution in the tubules of dentin substrate. In the proposed study, ion concentration of the remineralizing solution can be exactly halved in order to study effect of viscosity. Proposed concentration of remineralizing solution is given in Table 3.1
Table 3.1: Chemical composition of artificial saliva (remineralization) solution for future study

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KCl</td>
<td>0.4g/L</td>
</tr>
<tr>
<td>2</td>
<td>NaCl</td>
<td>0.2g/L</td>
</tr>
<tr>
<td>3</td>
<td>CaCl$_2$.2H$_2$O</td>
<td>0.181g/L</td>
</tr>
<tr>
<td>4</td>
<td>NaH$_2$PO$_4$.2H$_2$O</td>
<td>0.69g/L</td>
</tr>
<tr>
<td>5</td>
<td>Na$_2$S.9H$_2$O</td>
<td>0.0025g/L</td>
</tr>
<tr>
<td>6</td>
<td>NaN$_3$</td>
<td>0.1g/L</td>
</tr>
<tr>
<td>7</td>
<td>HEPES buffer</td>
<td>20 mmol</td>
</tr>
</tbody>
</table>

Concentration of the buffer is kept constant as it is required to keep the pH above 6.5.

3.1.3 ALD temperature

The atomic layer deposition was performed at 100 °C on the dentin substrate. This temperature was chosen so as to avoid any changes in the appearance of the substrate. Also, it is reported that chemical changes start to take place above 300 °C. It is reported in the literature that temperature above 42.5 °C can result in irreversible damage to the dental pulp leading to pulp necrosis. Thus, for in vivo studies, temperature of 100 °C is not suitable. Thus, it is also important to verify if ALD can successfully coat HAp surface with TiO$_2$ below the critical temperature of 42.5 °C [47].
3.2 ALD of Ca and P

3.2.1 Background

In the present project, TiO$_2$ nanolayer has been used to accelerate formation of CaP crystals on demineralized dentin. This study has proved that the human dentin has potential nucleation sites responsible for surface adsorption of precursor molecules in atomic layer deposition reaction leading to thin film formation. Based on these results, another approach to initiate remineralization of demineralized dentin could be coating the exposed surface with HAp nano-layer using atomic layer deposition.

Titanium and its alloys are widely used in the field of orthopedic surgery as implants, screws, and pins due to their strength, durability, and biocompatibility [68]. Various chemical treatments have been tried previously to coat these surfaces with calcium phosphate in order to promote bone bonding and bone formation. Stoichiometric Ca/P ratio of hydroxyapatite is close to that of the natural bone i.e. 1.67, [69, 70] resulting into bonding between deposited HAp and natural bone. Different techniques used before include pulsed laser deposition (PLD), sputtering, sol-gel, plasma-spraying etc. [69].

Recently in this decade, atomic layer deposition technique has been tried to coat such surfaces with calcium phosphate as it can produce highly conformal and uniform coating. HAp thin films have been deposited using ALD on various substrates such as Titanium, Silicon and bioglass. ALD of calcium phosphate can be tried on dentin surface so as to directly initiate the process of remineralization without the need of TiO$_2$ nanolayer.
Available literature suggests that the ALD of Ca is a fairly established process. The ALD chemistry of phosphorus has only been studied for the deposition of various phosphide thin films [71]. ALD of CaP is discussed in detail in this section.

3.2.2 Atomic Layer Deposition of Calcium Oxide

ALD of CaO has been reported in literature and is a fairly established process. Two Main categories of calcium precursors used are β-ketonates and cyclopentadienyls [69, 72, 73]. Whereas, the reported processes report Ca(thd)$_2$ type precursor from the family of β-ketonates (thd is 2,2,6,6-tetramethyl-3,5-heptanedione) and Ca(Cp$_3$i)$_2$ i.e. 1,2,4-(C$_3$H$_7$)$_3$C$_5$H$_2$Ca from the family of cyclopentadienyls [74]. The optimum temperature range provided for these 2 precursors is in the range of 200 °C to 300 °C.

Ca(thd)$_2$ precursor is used along with O$_3$ as an oxidizer to produce nanofilms of CaCO$_3$ [74]. Formation of pure oxide CaO is not reported. During this ALD process, firstly the surface gets saturated by Ca(thd)$_2$ monolayer followed by its oxidation with O$_3$ in order to get rid of the organic ligand thd which results in liberation of CO$_2$ and H$_2$O. Consequently, the surface is subjected to CO$_2$ atmosphere resulting in the formation of carbonate instead on pure CaO nanofilm [74]. The as-deposited CaCO$_3$ nanofilm is reported to be present in amorphous form. Thus post-deposition annealing treatment (at temperature >400 °C) is a promising technique giving crystalline film and pure CaO form is also obtained with this treatment.

Another process used Ca(Cp$_3$i)$_2$ as metal precursor with H$_2$O as an oxidizer [72]. This process has not resulted in the formation of metal carbonates but chemical analysis of the as-deposited nanofilm has suggested presence of hydroxides (Ca(OH)$_2$) along with pure oxide. This is attributed to moist atmosphere due to presence of water vapor as an oxidizer. CaO film has a
tendency to get oxidized easily. This procedure has also reported formation of amorphous nanofilm which requires post-treatment to increase crystallinity.

3.2.3 Atomic Layer Deposition of Phosphorus[71]

Few alkyl volatile phosphorus precursors are mentioned in the literature based on the ALD studies performed till now. Metal Phosphates are reported as phosphorus precursors for ALD on Al, Ti and Li phosphate thin films [75]. Volatile alkyl phosphorus precursors such as PH$_3$ and tBuPH$_2$ as well as phosphorus pentoxide P$_2$O$_5$ are reported precursors. Different ALD experiments performed for the deposition of phosphate thin films have reported (CH$_3$O)$_3$PO i.e. (Trimethyl phosphate TMPO) [76] as the phosphorus precursor which is also well-used for the ALD of calcium phosphate thin film which will be discussed in the next section.

3.2.4 Ca-P-O thin film using ALD

The objective behind Ca-P-O thin film coating is the formation of hydroxyapatite phase. Thus, an important factor to be considered is the crystallinity of the resulting film. Hydroxyapatite (crystalline) has Ca/P ratio of 1.67. The ultimate goal of thin film deposition is for its application in the biomimetic remineralization process. The main approach behind this deposition is to perform ALD with calcium precursor and phosphorus precursor in a cyclic manner so that the first monolayer formed by Ca will react with the later containing P and so on. Ca-P-O thin films are tried to form in this cyclic manner [70].
2 different established ALD processes are reported which were tried on silicon as well as titanium substrates:

3.2.4.1 Combined ALD process containing Ca and P source

Ca(thd)$_2$/O$_3$ and (CH$_3$O)$_3$PO/H$_2$O ALD processes are combined for formation of CaP thin film of nanolayer thickness [77]. Very narrow temperature window i.e. 275 °C - 300°C was observed for the ALD process. The first ALD run (Ca(thd)$_2$/O$_3$) is responsible for CaCO$_3$ monolayer formation. Next deposition is observed to take place by the exchange of CO$_3^{2-}$ and PO$_4^{3-}$ anions after the next ALD run of (CH$_3$O)$_3$PO/H$_2$O [70].

Use of Ca(Cp$_{3i}$)$_2$ as calcium source and H$_2$O as an oxidizer is also a potential technique. The drawback with this Ca source is that the films produced with this precursor have low carbonate content thus, incorporation of phosphate group (PO$_4^{3-}$) will be difficult in the as-deposited Ca thin film making formation of CaP thin film difficult.

3.2.4.2 CaO deposition followed by post-treatment

Another attempt for ALD of CaP was to form a thin film of CaCO$_3$ using Ca(thd)$_2$ and O$_3$ precursors. This as-deposited film is then reacted with dilute solution containing phosphorus (at 85 °C - 90 °C). Reported solution is DAP i.e. (NH$_4$)$_2$HPO$_4$ in DI water or 1 M NH$_3$ [69]. This chemical reaction did not take place at room temperature. Formation of amorphous CaP throughout the depth of as-deposited thin film was observed.

3.2.4.3 Drawbacks of the reported procedures

Optimization of process parameters can give pure CaP films but with Ca/P ratio which is far away from crystalline HAp. The thin films formed with ALD are amorphous in form thus post
deposition annealing at temperatures above 400 °C is reported in the literature. Annealing at higher temperatures has resulted in the formation of crystalline HAp with Ca/P ratio of 1.67 resembling that of natural HAp. The present literature findings are for the purpose of CaP deposition on Human dentin and ultimately teeth. Teeth contains different parts such as dentin, enamel and cementum which have different thermal expansion coefficients. Thus, exposing teeth to such high temperatures will greatly affect the mechanical stability of the substrate. Thus, there is a need to tune the ALD parameters in order to not exceed the allowed temperature range and obtain crystalline HAp.

3.2.4.4 Strategy to overcome temperature limitations[7]

A study has reported a strategy to work with precursors for Ca and P source and optimize there ALD cycle ratio in order to get required Ca/P ratio. To target the ratio of 1.67, Ca ALD subcycle is repeated 6 times before one P subcycle. This strategy has resulted in Ca/P ration of 1.61 which is close to that of the natural HAp. Consequently, it was possible to bypass the need of post-deposition annealing.

Further optimization of ALD subcycle ratio, temperature and other process parameters can result in the formation of HAp thin film on the substrate. In summary, as we have demonstrated that ALD of metal oxide is possible on human dentin, we can try to deposit CaP thin films by choosing appropriate precursors based on the available literature and tuning the ALD parameters. This as-deposited HAp film should be further able to restore lost minerals from the teeth and should resemble physicochemical characteristics of natural human dentin.
Chapter 4

Clinical Scope

After working on ALD process for hydroxyapatite matrix, it is required to provide the clinical scope and relevant applications of the research performed. The research mainly led to two findings. First was that the hydroxyapatite matrix provides sites for nucleation in atomic layer deposition. Secondly, the as-deposited TiO$_2$ nanofilm accelerated CaP growth by likely electrostatic force of attraction in biomimetic conditions. Hence, ALD of TiO$_2$ or Ca and P as proposed in the future work, can be an innovative solution to the problem of dental caries. The deposition can provide minerals to the demineralized matrix. Also, ALD can also provide minerals such as fluorine which are usually provided with conventional methods such as toothpastes. ALD has a potential to create next generation of remineralization techniques, as different materials can be provided to the dental matrix such as TiO, F etc.

The as-deposited TiO$_2$ has potential to attract minerals in order to remineralize dentin matrix. Moreover, the capability of TiO$_2$ nanofilm to provide mechanical stability can also be verified. Tooth varnish is a category of dental treatment in which the varnish is applied to the exposed areas to reduce sensitivity and strengthen the enamel and dentin. Thus, ALD can be a potential way of applying such treatment with uniformity.

This work was aimed to take the first step towards dental applications of atomic layer deposition technique and examine the conditions necessary for the desired reaction. The goal of the present work must be to optimize process parameters in order to make the technique possible at desire working and treatment conditions. Also, to work with clinicians to understand any flaws or complications in the clinical aspects of the technique is important so that desired solution to the
practical difficulties of the technique can be engineered. In summary, ALD on tooth hydroxyapatite has potential to solve dental problems such as caries, hypersensitivity and increase the strength of dental hard tissue.


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Professional Profile:
- Over 5 years of advanced academic and research experience in the field of chemical engineering and process technology
- More than 3 years of research experience in process engineering
- 6 weeks industrial internship in R&D with RPG Life Sciences Ltd. India

Work Experience
- Teaching Assistant in the department of chemistry, University of Illinois at Chicago Jan 2014 – May 2015
  - Grade and critic problem sets and homework, decide course assignments
  - Conduct review lectures and discussion sessions
  - Instruct a group of 25 students and assist in experiments
- Industrial Internship: Research and Development intern – Mumbai, India RPG Life Sciences (Rama Prasad Goenka Enterprises) May 2012- June 2012
  - Involved in analysis of product irregularities and quality control tests for shelf life determination of API. (HPLC, GC, FTIR)
  - Investigated production processes & optimized process parameters involved in catalytic chemical reactions leading to cost savings of $2 million
  - Assisted in development & implementation of new API processes and 100x reactor scale up (pilot plant) for optimization of parameters
- Chemical Manufacturing plant (API) (clean room experience)
  - Investigated unit operations such as distillation, filtration, granulation, powder processing
  - Assisted in process optimization calculations; Material balances for different process systems
  - Involved in scale up from pilot plant to production of chemical substances for commercial purposes
  - Experienced with utilization of six sigma methodology and Process Safety Management system

Education:
- Master of Science in Chemical Engineering (MS Thesis) Expected Aug 2015
  University of Illinois at Chicago, USA
- Bachelor of Technology in Pharmaceutical Sciences and Technology Aug 2009 – April 2013
  Institute of Chemical Technology (ICT) (Formerly known as UDCT), Mumbai, India

Technical Skills:
- Computer tools: C, C++, MATLAB, FORTRAN, R (Programming language for Statistics), Polymath, AutoCAD, MS office
- DOE software: Minitab, Modde, Design Expert
- Analytical Instruments: HPLC, GC, SEM, EDS, FTIR, Polarimeter, MS, UV/Vis Spectrophotometry, Goniometer
- Unit Operations, Process Equipment: Pumps, piping, valves, Roller Compactor, Compressor, Jet Milling, Spray Drying,
Research Experience

- Research student “Advanced Material Research Laboratory” - Chicago, IL (MS Thesis)  Jan 2014 - Present
  “Atomic Layer Deposition for infiltration of Ca hydroxyapatite by TiO$_2$ nanofilm”
  Advisor: Prof Christos Takoudis
  - Optimization of process variables for thin film deposition (correlate to ALD reactor parameters), monitoring nano-thickness
  - Characterization of infiltration by SEM, TEM and EDS techniques and statistical data analysis
  - Investigation of surface modifications (water contact angle & roughness) and heterogeneous nucleation for remineralization study

  - Developed a greener technique of immobilization for a stereospecific steroid biotransformation at C17 (beta isomer)
  - Characterization of batch process and 10x scale up batch
  - Optimization of process parameters, catalyst amount and reactor parameters for maximum throughput, capacity utilization & recovery of purest form of the end product with successful scale up
  - 30% cost savings in commercial production of 3 methyl ester of β- Estradiol with return of investment of 3.68 years

Academic projects

- Sedimenting viscous drop modelled as a swarm of Stokeslets  Jan 2014- May 2014
- Purification of proteins using separation techniques  Jan 2013
- Investigation of Pervaporation Technology for azeotrope separation  July 2012 - Nov 2012
- Ceramic Based Membrane Technology For Oxygen Separation  Jan 2012
- “Young Innovators Choice Competition| Industry defined problem, Indofil”  Jan 2012
  Developed 2 scalable and cost effective processes for regio-selective alkylation of 1h-1,2,4 Triazole

International Publication

Bela Joshi, Shirin Shinde, Ashish Khinvasara, Sumedh Joshi; “Cationic Polymers as Biodegradable Vectors for plasmid DNA Delivery” ;“Pharma Utility” Volume 7; Issue 3; Aug 2013

Professional presentations and conferences

- “Ceramic Based Membrane Technology For Oxygen Separation” ; Bela Joshi and Shirin Shinde, Exergy Poster Presentation, Mumbai, India, January 2012
- “PPO Copolymer for Efficient Gene Delivery for Cancer Treatment”; Bela Joshi, 2nd International Conference on Stem Cells and Cancer Research, Pune, India, October 2011 (Consolation Prize)

Leadership Experience:

- Editorial Head and Secretary of the Technological Research Journal at the Undergraduate Institute (July 2011 – April 2012)
- Event Head at the National Level Annual Cultural Festival of ICT (Jan 2012)

Honors and Awards:

- Active member of “Institute of Biomaterials, Tribocorrosion and Nanomedicine, Chicago”.
- Recipient of “B.Tech. Merit Prize” under the ICT Students’ Funds for Second year, Third Year and Forth year B. Tech for securing Second, Third and Third place respectively among all the 7 branches of B. Tech. (ranked in top 2% of the class).
- Awarded a scholarship from AICTE, India for all 4 years of undergraduate degree in chemical technology (2009-2012)