Glass Ionomer Cements with Improved Bioactive and Antibacterial Properties

SONG CHEN
Abstract

Dental restorative cements are placed in a harsh oral environment where they are subjected to thermal shock, chemical degradation, and repeating masticatory force. The ideal restorative dental cements should have superior mechanical properties, chemical stability, aesthetic, good handling properties, biocompatibility, antibacterial properties, and preferably bioactivity. This thesis presents research on dental restorative cements with enhanced properties. The overall aim was to increase the bioactivity and antibacterial properties of dental restorative cements without affecting their other properties.

The effect from adding calcium silicate to glass ionomer cement (GIC) was investigated. The results showed that calcium silicate could increase the bioactivity and reduce the cytotoxicity of conventional glass ionomer cement without compromising its setting and mechanical properties.

Hydroxyapatite (HA) with a high aspect ratio and thin nacreous-layered monetite sheets were also synthesized. Nano HA particles with an aspect ratio of 50 can be synthesized by both precipitation and hydrothermal methods. The aspect ratio was controlled via the pH of reaction medium. Thin nacreous-layered monetite sheets were synthesized through a self-assembly process in the presence of an amine based cationic quaternary surfactant. Temperature, pH, and presence of surfactant played essential roles in forming the nacreous-layered monetite sheets. Then the effect from adding silver doped HA and monetite particles was investigated. The results showed that the antibacterial properties of GIC could be increased by incorporating silver doped HA and monetite particles. Further examination showed that the pH change, F− ion release, and concentration of released Ag+ ions were not responsible for the improved antibacterial properties.

The quasi-static strengths and compressive fatigue limits of four types of the most commonly used dental restorations were evaluated. In our study, resin modified GIC and resin-based composite showed superior static compressive strength and fatigue limits compared to conventional GIC. The static compressive strength of dental cements increased with the aging time. However, aging had no effect on the compressive fatigue limit of resin modified GIC and resin-based composite. The compressive fatigue limit of conventional GIC even showed a drastic decrease after aging.

Keywords: biomaterial, glass ionomer cement, bioactivity, hydroxyapatite, monetite, calcium silicate

Song Chen, Department of Engineering Sciences, Applied Materials Sciences, Box 534, Uppsala University, SE-75121 Uppsala, Sweden.

© Song Chen 2016

ISSN 1651-6214
ISBN 978-91-554-9670-8
urn:nbn:se:uu:diva-301924 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-301924)
To my family
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


VI  **Chen, S.**, Gururaj, S., Xia, W., Engqvist, H. Synthesis of Ag doped calcium phosphate particles and their antibacterial effect as additives in dental glass ionomer cements. Accepted by *Journal of Materials Science: Materials in Medicine*

Reprints were made with permission from the respective publishers.
Author’s Contributions

Paper I: Major part of planning, experiment work and writing

Paper II: Part of planning and experiment work, major part of writing

Paper III: Major part of planning, experiment work and writing

Paper IV: Major part of planning, experiment work and writing

Paper V: Major part of planning, experiment work and writing

Paper VI: Major part of planning, experiment work and writing
Also published


### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU</td>
<td>Colony-forming unit</td>
</tr>
<tr>
<td>C₂S</td>
<td>Dicalcium silicate (Ca₂SiO₄)</td>
</tr>
<tr>
<td>C₃S</td>
<td>Tricalcium silicate (Ca₃SiO₅)</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>DCT</td>
<td>Direct contact test</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy dispersive X-ray</td>
</tr>
<tr>
<td>GIC</td>
<td>Glass ionomer cement</td>
</tr>
<tr>
<td>HA</td>
<td>Hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂)</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>Inductively coupled plasma atomic emission spectroscopy</td>
</tr>
<tr>
<td>MAAs</td>
<td>Metabolic activity assays</td>
</tr>
<tr>
<td>MTA</td>
<td>Mineral trioxide aggregate</td>
</tr>
<tr>
<td>PAA</td>
<td>Polyacrylic acid</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>SAXS</td>
<td>Small angle X-ray scattering</td>
</tr>
<tr>
<td>SBF</td>
<td>Simulated body fluid</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TSB</td>
<td>Tryptic soy broth</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
</tbody>
</table>
Introduction

The function and integrity of teeth can be destroyed by caries or trauma. After removing the caries or curing the trauma, dental restorative cements are required to restore the missing tooth structure. Dental restorations are placed in a rather harsh oral environment. They are subjected to thermal shock, chemical corrosion and repeating masticatory force, which require them to have superior mechanical properties. The ideal dental restorative material should have good biocompatibility in contact with the tooth and have optimal handling and setting properties. Moreover, as shown by other researchers\(^1,^2\), secondary caries become one of the most common reasons for the replacement of dental restorations nowadays. Therefore dental restorative materials with bactericidal properties have been a constant quest. The ideal dental restorative cements should also be bioactive, which could increase their bonding to teeth and close the gaps between them by forming an apatite layer\(^3\).

To fulfill the requirements listed above, materials such as amalgam, zinc oxide-eugenol, zinc polycarboxylate, glass ionomer cement (GIC), and resin composites have been used as dental restorative materials\(^4\). Among these materials, GIC is one of the most widely used dental restorative cements nowadays. Conventional GIC is based on the reaction between polyacrylic acid (PAA) and glass powder containing silica, calcium, alumina, and fluoride. GIC is considered as superior to other types of dental cements mainly due to its esthetics and fluoride release over a prolonged period of time. The disadvantages of GIC include its brittleness and sensitivity to moisture\(^5\). Moreover, GIC has no bioactivity due to the release of unreacted PAA and also a low pH\(^6\). In order to overcome these disadvantages, studies have been done to modify either the glass powder or polyelectrolyte. For example, metallic fillers such as Zn, Sr, and Ag are incorporated into the cements to increase their mechanical and antibacterial properties; new acrylic acid copolymers and amino acid containing polyelectrolyte have been used to replace the traditional PAA\(^7,^8\).

In this thesis, our work on developing GIC with enhanced properties is presented. Calcium silicate and calcium phosphate particles are synthesized and then used as additives in conventional GIC. The setting properties, mechanical properties, bioactivity, antibacterial properties, and biocompatibility of GIC are evaluated in this thesis.
The specific aims and objectives are presented in the following sections. In the background part, basic knowledge about dental restorative cements, glass ionomer cements, calcium silicate biomaterials, and calcium phosphate biomaterials are provided. In the section synthesis and characterization of calcium silicates and calcium phosphates particles, we present our work on preparing calcium silicate and calcium phosphate particles. The process of preparing cement samples is presented in the subsequent cement preparation section. The following part we evaluate the setting time, statistic compressive strength and fatigue performance, bioactivity, antibacterial properties, and cytotoxicity of GIC. The analytical techniques and methods used in this thesis are presented at the end.
Aims and objectives

The aim of the thesis was to develop enhanced GIC by incorporation of synthesized calcium phosphate and calcium silicate nanostructured particles. The desired properties include setting properties, mechanical strength, bioactivity, biocompatibility, and antibacterial properties.

The objective of Paper I was to increase the bioactivity of the conventional GIC by incorporating calcium silicate materials. The setting time, compressive strength, pH change, and in vitro bioactivity of the modified cements were also evaluated in this paper. The cytotoxic effect of the modified GIC was evaluated in Paper II. The ion concentrations were measured to correlate to the cytotoxicity results of GIC. The aim of Paper III was to evaluate the quasi-static compressive strength and the compressive fatigue limit of four types of the most commonly used dental restorative materials. The aging effect on the mechanical performance of these dental cements was investigated. The objectives of Paper IV and Paper V were to synthesize and characterize calcium phosphate particles with specific nano-structures, which could be acted as candidates for modification of GIC structures. In Paper IV, hydroxyapatite (HA) with a large aspect ratio has been synthesized through hydrothermal methods and precipitation methods. In Paper V, nacreous-like monetite sheets were synthesized by precipitation methods guided by a surfactant. The mechanism of forming the structure and its characterization are also discussed in the paper. The objective of Paper VI was to enhance the antibacterial properties of GIC by incorporating silver doped calcium phosphate materials. The ion concentrations (F⁻ and Ag⁺) and pH were measured to correlate to the antibacterial results.
Dental restorative materials

The use of dental materials by human beings dates back to 5000 years ago when Phoenicians used gold bands and wires. Since then a variety of natural materials such as ivory, shells, and stones were used to replace or restore the cavities in teeth. During the 18th and 19th centuries, synthetic materials such as gold foil and amalgam started to occur and were widely applied in clinics by dentists. More recently, inorganic dental cements, synthetic resins, composites, metal implants, and ceramics have been introduced into dentistry.

‘Cements’ in the field of construction materials usually means inorganic materials that can combine other components to form a strong building structure. Different from that in construction materials, ‘cements’ in dentistry has a broader definition and includes a larger amount of materials. Based on major chemical reacting components, dental cements include zinc phosphate, zinc oxide-eugenol (ZOE), zinc polycarboxylate, glass ionomer cement (GIC), resin cement, and mineral trioxide aggregate (MTA). Based on the applications, dental cements can be classified as cements for luting, cements for pulp protection, and cements for restoratives. Luting cements are materials placed between the tooth structure and the prosthesis to combine them together. Cements for pulp protection are to protect the tooth from pulp irritation, thermal shock, and microleakage. They can be further classified as liner, base, and varnish.

Dental restorative cements are used to restore the function and integrity of missing tooth structure. They can be further classified as immediate restorations and permanent restorations depending on the intended time periods. The most frequently used dental restorative cements nowadays are zinc phosphate cements, GICs, and resin composites. Ideal dental restorative cements should fulfill physical, chemical, and biological requirements. Concretely, these requirements include esthetic transparency, good handling properties, proper setting time, good mechanical properties, biocompatibility, and enhanced antibacterial properties. Requirements for dental restorative cements are specified in ISO 4049-2009: Dentistry-polymer-based restorative materials and ISO 9917-2007: Dentistry-water-based cements.
Glass ionomer cements (GICs)

Since they were invented by Wilson and Kent in 1969, glass ionomer cements (GICs) have been commonly used in dentistry. The advantages of GICs include good biocompatibility, esthetics, and fluoride release over a prolonged period. The GICs have been used clinically as restorative cements, luting cements, base and liner.

The curing of GICs is based on the reaction between polycarboxylic acid (mostly polyacrylic acid) and calcium-alumino-silicate glass. When the two components are mixed, Ca$^{2+}$ and Al$^{3+}$ ions from glass powder are leached into the aqueous solution after attacking by polycarboxylic acid. The polycarboxylic acid chains are firstly cross-linked by Ca$^{2+}$ and later Al$^{3+}$. A 3D network is formed after curing of the cements, see Figure 1.

Commercially available GICs usually contain two parts: powder and liquid. Originally, polyacrylic acid solution is the main constituent of the liquid components. Tartaric acid acts as an additive in most of the GICs to improve the handling properties, decrease the setting time, and improve the working time. New acrylic acid copolymers and amino acid containing polyelectrolytes are currently used to reinforce the GICs. In one special GIC, the polycarboxylic acid can be served as freeze-dried powder and be premixed with glass powder, the liquid component is water or tartaric acid solution.

The physical and handling properties of GICs are greatly influenced by factors such as molecular weight of polyacrylic acid, powder to liquid ratio (P/L), and size of the glass particles. Increases in the molecular weight have positive effects on mechanical strength. However, handling properties decrease with the increase of molecular weight. The properties can also be manipulated by changing the powder to liquid ratio. With the increase of powder to liquid ratio the mechanical properties increase while the handling properties decrease. The particle size of glass powder usually ranges from 15 µm to 50 µm. Cements with higher compressive strength can be obtained by using glass powders with a smaller particle size.

Recently, many attempts have been made to develop advanced GICs. Metal-reinforced GIC has been developed to improve the toughness of conventional GIC. Metallic fillers such as Zn, Sr and Ag are incorporated into the cements to increase their mechanical and antibacterial properties. Another type of reinforced GIC is resin-modified GIC, in which part of the liquid solution is replaced by methacrylate-based monomers. The resin-modified GIC cures by two mechanisms: light curing and chemical curing.
The resin-modified GIC usually exhibits superior mechanical properties to conventional GIC, but they show more shrinkage during setting. More recently, a calcium aluminate GIC has been introduced by replacing part of the glass powder with calcium aluminate. The calcium aluminate GIC shows good bioactivity and excellent mechanical properties.\textsuperscript{12}

Figure 1. Network of glass ionomer cement after curing.
Calcium silicate biomaterials

Calcium silicate biomaterials are well-known in construction materials because they are the main components of Portland cement. Calcium silicate biomaterials have attracted more and more attention since the invention of bioactive glass by Larry Hench. The first bioactive glass contains 46.1% SiO₂, 26.9% CaO, 24.4% Na₂O and 2.6% P₂O₅. It is able to bond to bone through an interfacial carbonated hydroxyapatite layer on the glass surface. Some of the compositions even show a soft tissue bond. Later, glass-ceramic apatite-wollastonite (A-W), which precipitates apatite and wollastonite, was invented by Kokubo. It shows higher bending strength and compressive strength than those of the human cortical bone. Moreover, it shows better bioactivity than synthetic hydroxyapatite. Those advantages made it widely used for spine prosthetics, replacing bone tumours, and coatings on hip prostheses.

The most well-known calcium silicate biomaterial in dentistry is ‘Mineral Trioxide Aggregate’ (MTA). It was first introduced to dentistry in 1995 and applied in endodontic in 1998. The composition of MTA is quite similar to Portland cement, which mainly consists of dicalcium silicate (Ca₂SiO₄, C₂S) and tricalcium silicate (Ca₃SiO₅, C₃S). Compared with Portland cement, there are no heavy metal constituents such as arsenic and lead, but it has additional radiopaque fillers to make the MTA distinguishable on a radiograph. ZrO₂, Bi₂O₃ and BaSO₄ have been used as the radiopaque fillers. The main advantages of MTA are its antibacterial properties due to its high pH when mixed with water and its sealing ability as well as its bioactivity. Now MTA has been used in endodontic applications such as root-end filling materials, pulp capping materials and pulpal revascularization protective materials.

There are three main types of calcium silicates: CaSiO₃, Ca₂SiO₄ and Ca₃SiO₅. CaSiO₃ has two phases: high temperature phase α-CaSiO₃ (pseudowollastonite) and low temperature phase β-CaSiO₃ (wollastonite). Amorphous CaSiO₃ transits to β-CaSiO₃ at 870 °C and β-CaSiO₃ transits to α-CaSiO₃ when the temperature is higher than 1125 °C. α-CaSiO₃ is unstable in room temperature so β-CaSiO₃ is the natural existent mineral phase. Both of α-CaSiO₃ and β-CaSiO₃ are nonhydraulic and the solubility of them in water is low. Unlike CaSiO₃, C₂S and C₃S are hydraulic and self-setting in water. When reacting with water, both C₂S and C₃S form C-S-H gel and Ca(OH)₂:
C\textsubscript{2}S, C\textsubscript{3}S \rightarrow \text{C-S-H} + \text{Ca(OH)}\textsubscript{2}

C-S-H provides most of the mechanical strength of Portland cement. It is largely amorphous and the structure is not well-recognized. Hydraulic production is high in alkaline due to the existence of Ca(OH)\textsubscript{2}\textsuperscript{34}. Although the hydraulic production and processing of C\textsubscript{2}S and C\textsubscript{3}S are similar, C\textsubscript{3}S hydrates faster than C\textsubscript{2}S and produces more heat during the hydraulic process. C\textsubscript{3}S is responsible for the early strength of Portland cement and C\textsubscript{2}S can continue to hydrate even after 28 days.
Calcium phosphate biomaterials

Calcium phosphate materials refer to minerals which contain calcium ions (Ca$^{2+}$) and orthophosphates (PO$_4^{3-}$), metaphosphates (PO$_3^-$), or pyrophosphates (P$_2$O$_7^{4-}$). The reason for using calcium phosphates as biomaterials is based on their chemical similarity to the inorganic composition of human bone mineral. Currently, calcium phosphate biomaterials are used as implant coatings, drug delivery agents, and scaffolds for bone regenerations. In this thesis we mainly discuss two types of calcium orthophosphates: hydroxyapatite (HA, Ca$_{10}$(PO$_4$)$_6$(OH)$_2$) and monetite (CaHPO$_4$).

HA is one of the most important calcium phosphate biomaterials. It is the main inorganic phase of human bone and teeth and has been made into granules, blocks, and scaffolds for the application of bone repair and regeneration $^{35,36}$. HA has a hexagonal crystal structure with a Ca/P ratio of 1.67. HA is the least soluble calcium orthophosphates under neutral conditions, see Figure 2. Therefore, the pH should be higher than 4 when synthesizing HA particles. The solubility of HA depends on the crystallinity, Ca/P ratio, and grain size. Higher crystallinity and larger grain size result in lower solubility. By decreasing the Ca/P ratio, calcium deficient HA can be obtained, which has larger solubility than stoichiometric HA $^{37}$. Ion substituted HA can be obtained by replacing Ca$^{2+}$, OH$^-$, and PO$_4^{3-}$ with other ions. For example, Ca$^{2+}$ ions can be substituted by ions such as Mg$^{2+}$ and Sr$^{2+}$, while OH ions can be substituted by F$^-$ and Cl$^-$. The solubility of HA as well as the chemical stability are affected by the type and quantity of substitution ions.
Figure 2. Solubility diagrams of calcium orthophosphates salts showing how the concentration of Ca$^{2+}$ changes with pH\textsuperscript{38}. Reprinted with the permission from the publisher.

Monetite (CaHPO$_4$) shows the least solubility among calcium orthophosphates under acidic condition (pH below 4.8), see Figure 2. CaHPO$_4$ has a triclinic crystal structure with the unit-cell dimension: $a = 6.90\text{Å}$, $b = 6.65\text{Å}$, $c = 7.00\text{Å}$, $\alpha = 96^\circ 21'$, $\beta = 103^\circ 54'$, $\gamma = 88^\circ 44'$. Synthetic monetite shows a rapid rate of bone formation which makes it a promising regeneration material in the orthopaedic and dental fields\textsuperscript{39,40}.
Synthesis and characterization of calcium silicate and calcium phosphate particles

Methods such as solid state reaction, co-precipitation, sol-gel method, mechanochemical, molten salt, hydrothermal method, etc. have been used to synthesize calcium silicates or calcium phosphate particles 41-47. By choosing different methods and changing the parameters, such as precursors, temperature, etc., particles with diverse morphologies and microstructures can be synthesized 36. In Paper I, the sol-gel method was used to synthesize wollastonite. In Paper IV, HA dots, rods, sheets, and fibers were synthesized through a precipitation method and a hydrothermal method. The mechanism to control the aspect ratio was discussed in the paper. In Paper V, the nacreous-like monetite was synthesized through a precipitation method. These HA and monetite could be acted as candidates for modification of GIC

Sol-gel method to synthesize wollastonite particles

As its name suggests, the sol-gel method usually involves colloidal-like solution and gel-like stages before obtaining the final particles. In this thesis, the colloidal-like solution was created by hydrolysis of tetraethyl orthosilicate (TEOS) using HNO₃ as the catalyst. Calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O) and ethanol were added after the complete hydrolysis of TEOS. The solution was then aged at 60 °C for 1 day for gelation and another day at 110 °C to remove the water and alcohol. The dried gel was calcined at 1000°C for 4h to obtain the final resultant powders. The synthesized powder had a spherical morphology observed by scanning electron microscope (SEM), as shown in Paper I. The X-ray diffraction (XRD) analysis showed that the particles were a mixture of wollastonite (β-CaSiO₃) and a small amount of larnite (Ca₂SiO₄). The existence of larnite was not a disadvantage, since both wollastonite and larnite are bioactive materials 48. The reason for using the sol-gel method instead of solid state reaction or mechanochemical routes is due to the lower sintering temperature and more homogeneous compositions of the sol-gel method 33. In addition, sol-gel derived particles, i.e. sol-gel-derived bioactive glass, show enhanced bioactivity because of their higher specific area and more nanoporous network 49, 50. In our study
we would like to increase the bioactivity of GIC by including wollastonite, therefore the sol-gel method is the most suitable in this case.

Precipitation and hydrothermal methods to synthesize hydroxyapatite with high aspect ratio

Both the precipitation method and the hydrothermal method are wet chemical methods. The difference is that the temperature and pressure used in the hydrothermal method is usually higher than that in the precipitation method. Therefore particles obtained by hydrothermal methods have better crystallinity than those obtained by precipitation methods. The disadvantage of hydrothermal methods is their higher equipment cost\(^{51,52}\). In addition, the hydrothermal process is conducted within sealed containers, which makes it difficult to observe and manipulate the reaction. In Paper IV, nano hydroxyapatite (nHA) dots, rods, sheets, and fibers were firstly synthesized through hydrothermal methods, see Figure 3. The XRD pattern confirmed that all the resultant particles were HA, see Figure 4. The essential point of obtaining HA with different aspect ratios was to control the pH. The aspect ratio increased with a decrease of the pH. At pH 11, 7.4, 6, the resultant particles were dots, sheets, and rods respectively. However, it was difficult to obtain nHA with a larger aspect ratio. As we mentioned above, when the pH is lower than 4.8, the least soluble phase is monetite, therefore when preparing fiber nHA using the hydrothermal method, some dicalcium phosphate dehydrate (DCPD) and anhydrous dicalcium phosphate (DCPA) might coexist in the solution. This was observed in our experiment and has also been reported by other researchers\(^{43}\). In addition, the morphologies of nHA fibers were not stable and varied from batch-to-batch. Therefore, to solve this problem, the precipitation method was chosen because the process of the precipitation method is simpler and easier to control. The aspect ratio of the nHA was also controlled by the pH. The fiber nHA with a large aspect ratio could be obtained with the existence of cetyltrimethylammonium bromide (CTAB). The resultant nHA particles by the hydrothermal and precipitation methods are summarized in Table 1.
Figure 3. SEM images of HA prepared by the hydrothermal method (a) pH = 11 (b) pH = 7.4 (c) pH = 6 (d) pH = 4. Reprinted from Paper IV with permission from the publisher.

Figure 4. XRD of HA prepared by the hydrothermal method. Reprinted from Paper IV with permission from the publisher.
Table 1. Summary of morphologies and aspect ratios of nHA

<table>
<thead>
<tr>
<th>Preparation method</th>
<th>pH</th>
<th>Morphology</th>
<th>Length</th>
<th>Diameter/width (nm)</th>
<th>Aspect ratio</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydrothermal</td>
<td>11.0</td>
<td>dots</td>
<td>10-25</td>
<td>1-3</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>hydrothermal</td>
<td>7.4</td>
<td>Sheets with some rods</td>
<td>50-100nm</td>
<td>25-75</td>
<td>1-3</td>
<td>110</td>
</tr>
<tr>
<td>hydrothermal</td>
<td>6.0</td>
<td>rods</td>
<td>50-200nm</td>
<td>10-30</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>hydrothermal</td>
<td>4.0</td>
<td>fibers</td>
<td>2-4µm</td>
<td>40-95</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>precipitation</td>
<td>5ml</td>
<td>NH$_3$·H$_2$O dots</td>
<td>10-20</td>
<td>1-3</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>precipitation</td>
<td>1ml</td>
<td>NH$_3$·H$_2$O dots</td>
<td>10-20</td>
<td>1-3</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>precipitation</td>
<td>0.8ml</td>
<td>NH$_3$·H$_2$O short rods</td>
<td>50-100nm</td>
<td>20-30</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>precipitation</td>
<td>0ml</td>
<td>NH$_3$·H$_2$O fibers</td>
<td>0.5-1µm</td>
<td>20-30</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

Precipitation method to synthesis nacreous like structures

Nacreous like structures are well-organized structures created by nature. In this structure, calcium carbonate tablets act as building blocks and the tablets are glued by elastic biopolymers produced by the nacre. The structure has attracted much attention due to its excellent toughness, stiffness, and impact resistance. In this thesis we synthesized monetite crystals with a thin nacreous structure using the precipitation method. A typical nacreous-like monetite obtained during our experiments was shown in Figure 5. The monetite sheets were 5–20 µm in width and 1 µm in thickness. Each monetite sheet showed a layered structure and the distance between each layer was around 2.6 nm from the small angle X-ray scattering (SAXS) experiment. Some flower-like monetite coexisted with the layered monetite with some of them covering the surface of the layered monetite. The XRD pattern showed that the particles were monetite, see Figure 5 (e). We further explored the mechanism involved in forming the structure and found that factors such as the temperature, initial pH, and amount of CTAB are important in forming the layered monetite. The parameters we investigated are shown in Table 2. The layered structure could not be achieved when the temperature was lower than 90 °C or without the existence of CTAB. High initial pH (pH = 11) was also required, however, Ca/P ratio was not the essential factor in forming these structures. More details can be found in Paper V. The process of forming the structure was time dependent. As we observed in the experiment, the plate-like monetite assembled to the stacked structure and after one hour the intact structure was formed. The concentration of the
precursor in the experiment was relatively high, so the particles in Figure 5 were not homogeneous. As the concentration of the precursors was decreased 30 times, uniform well-organized monetite particles can be formed, see Figure 6. The resultant monetite sheets were stacked with well-oriented small fibers. They were 10 µm long, 5 µm wide and 3 µm in thickness.

Figure 5. Characterization of typical monetite sheets synthesized at 90 °C, pH = 11 for 3h. (a-d) SEM micrographs. (e) X-ray diffraction pattern. (f) Synchrotron radiation result showing the distance between the layers is 2.6 nm. Adapted from Paper V with permission from the publisher.

Table 2. Parameter variations during preparation

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>CTAB (g)</th>
<th>pH</th>
<th>Ca/P (molar ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>1</td>
<td>11</td>
<td>1.30</td>
</tr>
<tr>
<td>90</td>
<td>0.3</td>
<td>11</td>
<td>1.30</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
<td>11</td>
<td>1.30</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>11</td>
<td>1.30</td>
</tr>
<tr>
<td>60</td>
<td>1</td>
<td>11</td>
<td>1.30</td>
</tr>
<tr>
<td>90</td>
<td>1</td>
<td>6</td>
<td>1.30</td>
</tr>
<tr>
<td>90</td>
<td>1</td>
<td>7.4</td>
<td>1.30</td>
</tr>
<tr>
<td>90</td>
<td>1</td>
<td>11</td>
<td>1.50</td>
</tr>
<tr>
<td>90</td>
<td>1</td>
<td>11</td>
<td>1.67</td>
</tr>
</tbody>
</table>
Figure 6. SEM micrographs of monetite sheets synthesized at a lower concentration of precursor solution (0.1M Ca(NO3)2•4H2O), stirring for 3h, Ca/P = 1.3. Reprinted from Paper V with permission from the publisher.
Cement preparation

The conventional glass ionomer cements used in Papers I, II, and VI were prepared on a plastic pad using a stainless spatula and then filled into the mold. We used two types of GIC as a control in paper I. One was water mixed GIC. In this type of GIC, reactive glass powder (SCHOTT, G018-090) and polyacrylic acid powder (Advanced Healthcare Ltd, Mw = 5000) were mixed as the powder part. The liquid was tartaric acid containing water. The other type of GIC was bought from Advanced Healthcare Ltd. In this type of GIC, the solution was polyacrylic acid and the powder was aluminosilicate-strontium glass powder. The latter one was also used as a control in Paper II and Paper VI. In Paper III, the Chemfil Rock capsule was mixed by the machine according to the manufactures’ instructions. The resin-based composite and light-cured resin reinforced GIC used in Paper III were placed into the molds and cured with a LED curing device (BlueLEX GT-1200) at 1000 mW/cm².

Samples for compressive strength and compressive fatigue limits measurement were 4 mm in diameter and 6 mm in height. After removing them from the mold, the specimens were polished using 800 grit silicon carbide paper. The specimens were then stored in distilled water at 37 °C until testing.

In the case of the bioactivity measurements (Paper I), the cements were stored in simulated body fluid (SBF) solutions. The volume of SBF (Vs) was calculated through the equation: Vs = Sa/10. Sa was the apparent surface area of the specimen. The SBF was replaced every day. After prefixed days, the samples were removed from the fluid, washed with deionized water and dried at 60 °C for later characterizations.

For the antibacterial measurement in Paper VI, cement samples with Φ = 10 mm, H = 1 mm were prepared and aged in distilled water for 1 day or 7 days.

For the cytotoxicity measurements in Paper II, cement disc samples with Φ = 12 mm, H = 2 mm were prepared and set in air for 3 hours. The cement discs were then stored in 0.5% NaCl solution for 7 days. Cement extracts were prepared by immersing a set cement disk in 1 ml of complete media. The surface-to-volume ratio is 3 cm²/ml, according to the requirement of ISO standard ISO-10993-11 54.
Setting time

The ideal restorative dental cements should have an appropriate setting time that is not too short or too long. It shouldn’t be too short so the dentists have enough time to manipulate it, while it shouldn’t be too long otherwise the patients are required to wait for a long time in the clinic. The definitions and measurements of setting time are varied according to different standards. In this thesis, the Gilmore needle method \(^{55}\) was used to determine the setting time. Both initial and final setting time can be measured by this method, depending on the mass and the tip diameter of the needles. More details about the Gilmore needle method can found in the *analytical techniques and methods*.

The initial setting time of the GIC control was around 240 s and the final setting time was around 300 s, as shown in Paper I. Addition of wollastonite slightly increased the initial setting time but had no effect on the final setting time, even when the wollastonite was up to 30%. The addition of MTA slightly changed the setting of GIC cements and additional tartaric acid was required to form cements with good handling properties. With 10% and 20% MTA, only the final setting time was slightly prolonged. While with 30% MTA, the initial and final setting time was prolonged to 570 s and 900 s respectively, which indicates that the network of GIC can be destroyed by adding too much MTA. This can be attributed to the setting mechanism of the GIC. The polyacrylic acid was quite acidic while the MTA was high in alkalinity. Therefore the mixing of the two components is accompanied by a fierce reaction which destroyed the network of GIC. Tartaric acid, as suggested by other researchers \(^{56, 57}\), can act as an accelerator in GIC which helps with the extraction of ions from glass and also acts as strong retardant for the hydration of Portland cement. Therefore, the tartaric acid might slow down the reaction between the PAA and MTA, which makes the addition of MTA into the GIC possible.
Dental restorations placed in an oral environment are subjected to repeating masticatory force. Therefore mechanical properties are important for dental restorations especially for those used under stress areas. In general, mechanical properties of dental restoration include elastic modulus, hardness, compressive strength, tensile strength, shear strength, flexural strength, fracture toughness, fatigue limits etc. These properties characterize the performance of the dental restorations under different applied force and provide basic data to predict their performance in the clinic. GICs, like other types of ceramics and cement, have high compressive strength but low tensile strength and fracture toughness. The minimum compressive strength required in ISO 9917 (2007) are 50 MPa for base/lining and 100 MPa for restorations. Therefore, it is important that we should also evaluate the mechanical properties when modifying the GIC. In this thesis, we mainly discuss the compressive strength and compressive fatigue limits of the cements. The discussions include the following:

1. Quasi-static and compressive fatigue performance of four types of dental restorations (Paper III)
2. Addition of calcium silicate materials on the compressive strength of GIC (Paper I)
3. Addition of Ag-HA and Ag-DCPA on the compressive strength of GIC (Paper VI)

Quasi-static and compressive fatigue performance of four types of dental restorations

Different from static compressive strength, in which an ultimate strength is applied to the cements, in compressive fatigue limits measurements, a low but cyclic load is applied to the cements. S-N plots (where S represents stress amplitude and N represents cycles to failure) and the staircase method are the most common methods to measure the fatigue limits. The staircase method requires fewer samples than that of S-N plots and the data analysis is simple. Therefore the staircase method was chosen in this thesis when evalu-
ating the fatigue limits. Further details about the staircase method and the experiment setting can be found in analytical techniques and methods part. In Paper III, we evaluated the quasi-static compressive strengths and compressive fatigue limits of four types of the most commonly used dental restorations, as well as the aging effect on those materials. The dental cements chosen for this study included: A conventional GIC (Fuji IX GP; IG), a zinc-reinforced GIC (Chemfil rock; CF), a light curable resin-reinforced GIC (Fuji II LC; LC), and a resin-based composite (Quixfil; QF). They are the most commonly used restorative dental cements in the clinic nowadays. The compositions of the products are shown in Table 3.

Table 3. Dental cements used in the fatigue measurements

<table>
<thead>
<tr>
<th>Name</th>
<th>Code</th>
<th>Type</th>
<th>Composition</th>
<th>Lot number</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC Fuji IX GP</td>
<td>IG</td>
<td>Conventional GIC</td>
<td>Polyacrylic Acid, Fluro-Alumo-silicate Glass</td>
<td>1403071</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polyacrylic Acid, 2-Hydroxyethylmethacrylate (HEMA), Urethane Dimethacrylate (UDMA), Fluro-Alumo-silicate Glass</td>
<td></td>
</tr>
<tr>
<td>GC Fuji II LC</td>
<td>LC</td>
<td>Light-cured resin-reinforced GIC</td>
<td>Polymethylmethacrylate, Itaconic Acid, Zinc Modified Fluro-Alumo-silicate Glass</td>
<td>1402081</td>
</tr>
<tr>
<td>Chemifil rock</td>
<td>CF</td>
<td>Zinc-reinforced GIC</td>
<td>Urethane Dimethacrylate (UDMA), Triethylene Glycol Dimethacrylate (TEGDMA), Di- and Trimethacrylate resins, Carboxylic acid modified Dimethacrylate, Silinated strontium aluminum sodium fluoride phosphate silicate glass</td>
<td>1310002004</td>
</tr>
<tr>
<td>Quixfil</td>
<td>QF</td>
<td>Resin-based composite (86% Filled By Weight)</td>
<td>Urethane Dimethacrylate (UDMA), Triethylene Glycol Dimethacrylate (TEGDMA), Di- and Trimethacrylate resins, Carboxylic acid modified Dimethacrylate, Silinated strontium aluminum sodium fluoride phosphate silicate glass</td>
<td>1408000913</td>
</tr>
</tbody>
</table>

The results showed that resin-based composites had the highest static compressive strength as well as the highest fatigue limits, followed by light curable resin-reinforce GIC and conventional GIC, see Table 4. This is in accordance with the literature\textsuperscript{59, 60}. The zinc-reinforced GIC, although is claimed to have improved fracture toughness by the manufacturer, showed similar compressive strength to that of conventional GIC. As stated by previous researchers\textsuperscript{61-63}, aging greatly affects the mechanical properties of GIC. Our results showed that aging increased the static compressive strength of all the tested dental cements. However, the aging effects on the fatigue limits were more complicated. The fatigue limits of resin-reinforced GIC and resin composites were not affected by the aging, while aging has a negative effect...
on the fatigue limits of conventional and zinc reinforced GIC, as shown in Table 4.

Table 4. Static compressive strength and compressive fatigue limit of test samples

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Compressive strength (MPa)</th>
<th>Compressive fatigue limit (MPa)</th>
<th>CFL/CS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IG (1d)</td>
<td>155 (8.3) a</td>
<td>62 (11.9) b</td>
<td>40.0</td>
</tr>
<tr>
<td>LC (1d)</td>
<td>168 (8.5) a</td>
<td>92 (6.6) c</td>
<td>54.8</td>
</tr>
<tr>
<td>CF (1d)</td>
<td>156 (21.8) a</td>
<td>61 (4.2) b</td>
<td>38.1</td>
</tr>
<tr>
<td>QF (1d)</td>
<td>244 (13.0) b</td>
<td>134 (7.8) d</td>
<td>54.9</td>
</tr>
<tr>
<td>IG (30d)</td>
<td>203 (21.8) c</td>
<td>39 (4.2) a</td>
<td>19.2</td>
</tr>
<tr>
<td>LC (30d)</td>
<td>217 (13.8) c</td>
<td>90 (4.2) c</td>
<td>41.5</td>
</tr>
<tr>
<td>CF (30d)</td>
<td>196 (14.1) c</td>
<td>42 (6.9) a</td>
<td>21.4</td>
</tr>
<tr>
<td>QF (30d)</td>
<td>300 (5.1) d</td>
<td>139 (21.7) d</td>
<td>46.3</td>
</tr>
</tbody>
</table>

Test groups with the same letter are not significantly different at P<0.05 level (one-way or two-way ANOVA, Tukey’s test).

Addition of calcium silicate materials on the compressive strength of GIC

Additives incorporated into the GIC can be divided into two types: inert additives which play the role of fillers and active additives which can react with the GIC component. The wollastanite can be considered as an inert filler. When the glass was replaced proportionally by wollastanite, the cross-link between the glass powder and polyacrylic acid decreased. This did not dramatically decrease the compressive strength if a small proportion (10% and 20%) of glass was replaced, see Figure 7. However, larger amounts of replacement decreased the compressive strength. When 30% wollastonite was added, the compressive strength was decreased. MTA contains two hydraulic calcium silicates: dicalcium silicate and tricalcium silicate. Unlike wollastanite, dicalcium silicate and tricalcium silicate are hydraulic and their hydraulic production is high in alkaline. When mixed with polyacrylic acid, MTA strongly reacts with GIC which generate lots of heat and result in inconsistency in the cements, which is not desired for dental cements. As mentioned above, tartaric acid is an important additive to facilitate the handling properties and increase the mechanical properties. In addition, it can delay the hydration of MTA. When the amount of MTA was up to 20%, tartaric acid was required in order to form good paste. When more MTA was incorporated more tartaric acid was required. It is interesting that when 10% and 30% MTA were added, the compressive strength dramatically decreased. However, 20% MTA doesn’t affect the compressive strength. The reason for this is unknown. More interestingly, the compressive strength of MTA modified GIC increased quickly during the two week’s storage in water. After 14 days, the compressive strength was comparable with conventional GIC.
Addition of Ag-HA and Ag-DCPA on the compressive strength of GIC

The Ag-HA and Ag-DCPA can be considered as inert fillers when replacing part of the glass powder. Our study showed that the addition of 10% and 20% Ag-HA and Ag-DCPA had no effect on the compressive strength of GIC, see Figure 8.

Figure 7. Compressive strength of GIC with wollastonite and MTA. Test groups with the same superscript letter are not significantly different at P<0.05 level (one-way ANOVA, LSD’s test). Reprinted from Paper I.
Figure 8. Compressive strength of the cements after storage in water for 1 day. There is no significant difference among the groups (one-way ANOVA, Tukey’s test). Adapted from Paper VI.
Bioactivity

Bioactive materials here are defined as materials that can promote the formation of hydroxyapatite mineral on the surface of materials\(^4\). Upon contact with body fluid, these materials form a hydroxyapatite interlayer between the tooth/bone structure and the filling materials so it can close the gaps between the materials and tooth/bone, as well as enhance the bone/tooth integration with the restorations. Therefore, bioactive dental materials with improved properties are highly needed. However, conventional GIC has been proved to have no bioactivity because of the release of unreacted PAA, which prevents the formation of hydroxyapatite on the GIC surface\(^6\). As we mentioned in the introduction, calcium silicate materials have demonstrated good bioactivity both in vitro and in vivo. The mechanism of apatite formation on calcium silicate materials can be described as follows\(^{64,65}\): when the calcium silicate materials are immersed in SBF solution, Ca\(^{2+}\) ions are leached and exchanged with H\(^+\) in the solution to form \(\equiv\text{Si-OH}\) group. The \(\equiv\text{Si-OH}\) functional group finally forms negatively charged \(\equiv\text{Si-O}\(^-\)\). Then Ca\(^{2+}\) ions in the SBF solution are attracted by the negatively charged \(\equiv\text{Si-O}\(^-\)\), which results in a positive charge of the materials’ surface. The positively charged compound attracts the PO\(_4\)\(^{3-}\) in return and triggers the formation of hydroxyapatite on the surface of calcium silicate materials. In the thesis, our interest is to investigate whether we can form a bioactive dental material based on the combination of GIC and calcium silicates. In Paper I, both non self-setting (wollastonite) and self-setting (MTA) calcium silicates were chosen to enhance the bioactivity of GIC. The hypothesis was that the modified GIC would have an enhanced bioactivity and comparable mechanical properties with conventional GIC.

In vitro bioactivity tests can be done in different physiologic solutions such as simulated body fluid (SBF), saliva, and phosphate-buffered saline (PBS) solution. All bioactivity tests in Paper I were done in the Kokubo's SBF solution. The Kokubo's SBF solution was prepared according to the literature\(^{66}\). The ion concentration of Kokubo's SBF and human plasma are listed in Table 5.
Table 5. Ion concentrations of the simulated body fluid and human blood plasma$^{67}$.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simulated body fluid (SBF)</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>142.0</td>
</tr>
<tr>
<td>K$^+$</td>
<td>5.0</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>2.5</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>147.8</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>4.2</td>
</tr>
<tr>
<td>HPO$_4^{2-}$</td>
<td>1.0</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Conventional GIC samples showed no formation of hydroxyapatite on their surface after immersion in SBF, see Figure 9. GIC with 10%, 20%, and 30% wollastonite showed a hydroxyapatite layer on the surface, indicating that the addition of wollastonite can enhance the bioactivity of GIC. The incorporation of MTA had a similar effect, see Figure 10. The formation of a hydroxyapatite layer was further confirmed by Energy dispersive X-ray (EDX) results, see Figure 11. Samples with wollastonite or MTA showed higher Ca and P peaks after immersion in SBF for 7 days. The most direct method to observe the hydroxyapatite on the surface of the material is grazing incidence X-ray diffraction. However, this technique is sensitive to the flatness of the surface. We didn’t observe any peak from grazing incidence X-ray diffraction pattern, probably due to the rough surface of the cements.

The increased bioactivity by wollastonite and MTA can be attributed to the increased pH and the bioactivity of calcium silicate materials. GIC is acidic while the incorporation of wollastonite or MTA can increase the pH of setting cements, either in water or in SBF solution, see Figure 12. It has been proved that higher pH benefits the apatite nucleation since apatite solubility decreases at basic pH$^{68,69}$. The Si-OH groups from wollastonite or MTA could be another reason for the enhanced bioactivity. As mentioned above, the Si-OH groups can facilitate the nucleation of apatite and the apatite continues to grow in the SBF solution after nucleation.
Figure 9. SEM images of the cements with wollastonite after soaking in water for 1 h (a) GIC (b) 10% wollastonite (c) 20% wollastonite (d) 30% wollastonite, and in SBF for 7 days (e) GIC (f) 10% wollastonite (g) 20% wollastonite (h) 30% wollastonite. Adapted from Paper I.
Figure 10. SEM images of the cements with MTA after soaking in water for 1 h (a) GIC (b) 10% MTA (c) 20% MTA (d) 30% MTA and in SBF for 7 days (e) GIC (f) 10% MTA (g) 20% MTA (h) 30% MTA. Adapted from Paper I.
Figure 11. Energy dispersive X-ray spectroscopy (EDX) elemental analysis: (a) GIC (b) 20% wollastonite (c) 20% MTA. The specimen was immersed in the SBF for 7 days at 37°C. Reprinted from Paper I.

Figure 12. pH changes of the solutions over a period of 7 days. GIC with (a) Wollastonite in SBF (b) Wollastonite in water (c) MTA in SBF (d) MTA in water. Reprinted from Paper I.
Antibacterial properties

Secondary caries is one of the most common reasons for the replacement of dental restorations. Therefore dental cements with good antibacterial properties which can reduce the risk of secondary caries are highly desired. Some efforts have been made to minimize the risk of secondary caries, including incorporation of soluble/releasing antibacterial agents, polymerizable antibacterial components or inorganic fillers. These strategies show some disadvantages, e.g. resulting in inferior mechanical properties, porous structures or increased cytotoxicity \(^{70-73}\). **Paper VI** presents our work on developing antibacterial GIC by using silver doped calcium phosphate particles as additives. Two types of calcium phosphates - silver doped monetite (Ag-DCPA) and silver doped hydroxyapatite (Ag-HA) were synthesized. The synthesis and characterization of these particles have been discussed above. The focus of this section is their antibacterial properties as additives in conventional GIC.

Methods such as counting colony-forming unit (CFU) and metabolic activity assays (MAAs) have been used to evaluate the antibacterial properties of dental cements. In this study, the direct contact test (DCT) was used. In the direct contact test, all of the bacteria are allowed to stay on the samples and proliferate. A bioluminescent strain, staphylococcus epidermidis Xen 43 was selected as the bacteria model. Viability of the bacteria can be evaluated through the intensity of luminescence, which is proportional to the number of viable bacteria. Poly(methyl methacrylate) (PMMA), which is considered as an inert material, was chosen as the control. The details regarding the bacterial viability assays can be found in *the analytical techniques and methods*.

There are some debates on the antibacterial effects of GIC. Some researchers show that the GICs have low antibacterial effects while others find that GICs possess antimicrobial properties due to low pH or fluoride release \(^{74, 75}\). In this study, the number of bacteria for conventional GIC was much less than for PMMA, indicating that conventional GICs possess antibacterial properties, see *Figure 13*. Compared with samples aged for 1 day, all the samples aged for 7 days showed a larger amount of bacteria, indicating that the antibacterial effect decreased with storage time. In case of samples aged for 1 day, samples with Ag-HA and Ag-DCPA showed fewer bacteria than the GIC control sample during the first three hours. However, the difference was not evident after four hours, see *Figure 13* (a). The antibacterial effects
of Ag-HA and Ag-DCPA were more evident for samples aged for 7 days, as all the samples with Ag-HA and Ag-DCPA showed smaller amounts of bacteria than the GIC control, see Figure 13 (b). These results showed that the antibacterial effect of GIC could be improved by incorporating Ag-HA and Ag-DCPA. We further studied pH, concentrations of Ag\(^+\) and F\(^-\) to see whether the improved antibacterial properties can be attributed to these factors. As shown in Figure 14, the F\(^-\) release dramatically decreased with time. Incorporation of Ag-HA or Ag-DCPA did not show negative or positive effects on the fluoride release, except for the samples with 20% HA, which showed higher F\(^-\) release on the day 3. No F\(^-\) was detectable on day 7. The concentrations of Ag\(^+\) for all the samples were under detection limit. pH was 4.9 after one day and it increased to 5.8 after 7 days’ immersion for the GIC control sample. The pH of the storage solution was not affected by the incorporation of Ag-HA or Ag-DCPA.

Figure 13. Direct contact measurement of the cements aged for 1 day (a) and 7 days (b). Each point on the curve is the average of five measured wells. Reprinted from Paper VI.
Figure 14. Fluoride release from different samples. * indicates significance (p < 0.05) between 20% Ag-HA and all other groups. Adapted from Paper VI.
Dental restorative cements are in contact with tooth tissue and physiological fluids, therefore the ideal dental cements should be biocompatible throughout their lifetime. Cytotoxicity testing is one method to evaluate the biocompatibility of biomaterials in vitro. Compared with animal model tests and clinical testing in humans, the cytotoxicity test in cell cultures is more efficient and easy to operate. Generally, there are direct and indirect measurements for the cytotoxicity test, depending on whether the cells are exposed to materials (direct) or extracted solutions of the materials (indirect). In this study, we chose indirect measurement to evaluate the cytotoxicity of GIC before and after modification. The details of this experiment can be found in Paper II.

Odontoblast-like cells, which are responsible for the formation of dentin and collagen-based mineralized tissue, were chosen for the cytotoxicity study. As previously reported by other researchers, conventional GIC shows cytotoxicity. In our experiment, conventional GIC showed the most cytotoxic effect, especially with 100% extract, see Figure 15. The cytotoxicity was reduced when the extract was diluted 20% and 50%. MTA was the least cytotoxic cement and there were no significant difference among the 20%, 50%, and 100% extracts. The results showed that incorporation of MTA or wollastonite reduced the cytotoxicity of GIC. In indirect tests, pH of the media, concentrations of inorganic ions, and organic compounds might affect the results of cytotoxicity studies. Thus pH in the extracts and the concentrations of Ca$^{2+}$, Al$^{3+}$, Sr$^{2+}$, and Si$^{4+}$ were measured. In the case of pH, GIC showed the lowest value (6.36) compared to other groups while MTA showed the highest (8.62). The previous studies have shown that a pH higher than 6.0 did not lead to significant cell death of human gingival fibroblasts; another study showed pH variation between 6 and 7 didn’t significantly affect the viability of fibroblasts and osteoblasts after 1 and 7 days. Therefore the differential of pH could not be identified as the main reason for the different cytotoxicity effects of the cements. In terms of ion releases, GIC showed the highest concentration of Si$^{4+}$ (19.9 mg/L) and Sr$^{2+}$ (100.3 mg/L), see Figure 16. However, as shown in a previous study, Sr$^{2+}$ was not toxic below 880 mg/L. Thus the concentrations of Si$^{4+}$ and Sr$^{2+}$ are too low to be toxic. As GIC showed the lowest the concentrations of Ca$^{2+}$ and a comparable concentration of Al$^{3+}$ to MTA, the cytotoxicity effect of GIC was unlikely caused by Ca$^{2+}$ and Al$^{3+}$. F$^-$ is also a contributory factor in the cyto-
toxic effects on odontoblasts. While the concentration of F\(^-\) was under the detection limit (0.02 mg/L), the negative effect of F\(^-\) was excluded. Based on the results, we concluded that the cytotoxicity of GIC was unlikely to be caused by release of inorganic ions (Ca\(^{2+}\), Al\(^{3+}\), Sr\(^{2+}\) and Si\(^{4+}\)) or low pH. Organic compounds such as polyacrylic acid might be released into the media during the storage time, it may be speculated that the cytotoxic effects of GIC were caused by these organic compounds. One possible reason is that the incorporation of wollastonite and MTA in GIC reduced the release of organic compounds from the GIC, decreasing the cytotoxicity of the modified GICs.

\[ \text{Figure 15. Viability of odontoblast-like MDPC-23 cells cultivated for 24h and 72h in MTA, GIC, M-mGIC, and W-mGIC extracts. The extracts were used undiluted (100%), 2-fold diluted (50%), and 5-fold diluted (20%). When not the same, letters indicate statistical differences (p < 0.05) between cement formulations at each cultivation time, capital letters represent 24h incubation and non-capital letters represent 72h incubation. C refers to negative control (fresh media) and C+ refers to positive control (0.1% triton). Reprinted from Paper II with permission from the publisher.} \]
Figure 16. Ion concentration of different samples (mg/L) considering (a) Ca^{2+}, (b) Al^{3+}, (c) Sr^{2+}, and (d) Si^{4+}. Test groups with the same letter are not significantly different at p < 0.05 level. Reprinted from Paper II with permission from the publisher.
Conclusions

The aim of this thesis was to develop enhanced dental restorative cements, mainly GICs, by incorporating synthesized calcium phosphate and calcium silicate particles in order to enhance the bioactivity, reduce the cytotoxicity, and improve antibacterial properties while keeping the mechanical strength.

Addition of calcium silicate based ceramics could increase the bioactivity of the material via formation of apatite on the surface without compromising their setting and mechanical properties. In addition, the cytotoxicity of conventional GIC could be moderated by incorporating these calcium silicate based ceramics. The cytotoxicity of GIC was unlikely to be caused by the release of inorganic ions or the low pH. We speculated that the incorporation of calcium silicate based ceramics reduced the total amount of organic compound present in the cement and thus decreased the cytotoxicity of the modified GICs.

The synthesis of calcium phosphates and calcium silicates were generally based on wet chemical processes. Nano hydroxyapatite with a high aspect ratio was synthesized by both precipitation and hydrothermal methods. Altering the pH of the starting solutions could control the aspect ratio in a repeatable way. Decreasing the pH resulted in an increase in aspect ratio. Nano hydroxyapatite particles with an aspect ratio of 50 can be synthesized by both precipitation and hydrothermal methods. Thin nacreous-layered monetite sheets were synthesized through a self-assembly process in the presence of an amine based cationic quaternary surfactant. The nacreous-layered monetite sheets can be stacked either from nano sheets or fibers. Our findings showed that a nacre-like structure can only be formed at high temperature (90°C), high initial pH (11), sufficient stirring time (3h), and under the presence of CTAB.

Ag-DCPA and Ag-HA particles were synthesized through precipitation method as we mentioned above. Our results showed that conventional GICs possess antibacterial effects and their antibacterial properties could be improved by incorporation of Ag-DCPA and Ag-HA particles. The results showed that the enhanced antibacterial properties were unlikely due to the pH change, F⁻ ion release or concentration of Ag⁺ release, probably caused by the direct contact of bacteria with Ag-DCPA and Ag-HA particles exposed on the surfaces of modified GIC samples.
Understanding the compressive strength and compressive fatigue performance of dental cements is important for the study of composite dental cements by incorporating nano bioactive ceramics. We evaluated the quasi-static strengths and compressive fatigue limits of four types of the most commonly used dental restorations, as well as the aging effect on those materials. The results showed that resin modified GIC and resin-based composite had superior mechanical properties than conventional GICs. Aging could increase the static compressive strength of dental cements but not the compressive fatigue limit. The compressive fatigue limit of conventional GICs even showed a drastic decrease after aging.
In this thesis, we have seen the possibility of enhancing dental cements by adding bioactive ceramic nanoparticles. Future work can be done to expand on the current findings. There are still many open questions, allowing for interesting work worthy of further development and discussion.

In the thesis we have demonstrated the possibility to combine the advantages of GICs and calcium silicate materials. The optimization of this process needs further consideration. In addition, polyacrylic acid could be replaced by other polycarboxylic acids to obtain dental cements with better handling properties. More comprehensive characterizations are required to evaluate the modified GIC, for example the bond strength between the teeth and restoratives, tensile strength of the modified cements, etc. should all be considered further. The compressive strength of the control GIC we used was around 120 MPa, developing bioactive GIC with higher compressive strength will also be interesting.

The relations between materials’ composition, structure and function are central to materials science. We have showed that the morphologies and structures of calcium phosphate particles can be controlled at the nano and micro-meter level. A more interesting question would be whether these calcium phosphate particles could self-assemble at a larger scale. Bulk materials with hierarchical nano structures usually exhibit superior mechanical properties. Therefore, it would be interesting to investigate the methods required in order to make the nano particles overcome the energy barrier so that they can assemble to larger scale structures.
**Analytical techniques and methods**

**X-ray diffraction**

The XRD measurement is based on Bragg’s law:

\[ n\lambda = 2dsin\theta \]

\( \lambda \) is the wavelength of the incident X-ray, \( d \) is the lattice planes distance, \( \theta \) is the angle between the incident X-ray and the measured lattice plane. There are lots of lattice planes in a crystalline material. The lattice planes which satisfy Bragg’s law result in constructive interference of the incident X-ray. By identifying several lattice planes of a material and comparing them with standard X-ray diffraction patterns, the phase composition of the crystalline material can be determined.

XRD can be used to identify powder or thin film. In this thesis, powder diffraction with a Bragg Brentano theta to theta setup was selected. Cu-K\( \alpha \) X-rays with a wavelength of 0.154 nm was used as the irradiation. The measurement was performed on a D5000 or D8 diffractometer (Bruker).

**SEM**

In SEM, electrons interact with the materials and the signals are collected to gain the materials’ surface topography and compositions. The signals can be secondary electrons which are generated from the collision of incident radiations and the loosely bonded electrons of materials, or backscattered electrons, which are generated from the elastic interaction between incident radiations and nuclei of atoms. Atoms with high atomic numbers backscatter electrons more strongly than those with low atomic numbers. Thus backscattered electrons are often used to analyze samples with different chemical composition contrast. In this thesis, secondary electrons were used as the signals while acquiring the SEM pictures.

The microscope used in the thesis was Zeiss LEO 1550. The samples were coated with thin gold/palladium layer to avoid charging of the samples.
Static compressive strength

The static compressive strength is measured by applying an ultimate force until the samples break. The compressive strength ($\sigma$) is calculated according to the formula:

$$\sigma = F/A$$

$F$ is the ultimate applied force and $A$ is the stress area of the applied force. All measurements of static compressive strength were conducted on a universal testing machine (Autograph AGS-X, Shimadzu). The crosshead speed was 1mm/min.

Staircase method

Staircase method was used to analyze the fatigue limits of the dental cements. For this method, an initial stress level was chosen and one sample was measured under this stress level. If the sample was broken at the stress level, then the next sample would undergo lower stress level; if the sample was intact at the stress level, the next sample would undergo higher stress level. An example is shown in Figure 17:

If the sample 1 was tested under the stress level 106 MPa and was intact, then the sample 2 would be tested under 114 MPa. Since sample 2 was broken, sample 3 would be tested under 106 MPa. The measurement continues until certain amount of samples was measured.

Figure 17. An example showing the process of staircase method

In the thesis, 17 samples were tested for each group and the run-out was set to $10^5$ cycles.

The compressive fatigue limit (CFL) and its standard deviation (SD) were calculated according to the following formula:

$$CFL = S0 + d\left(\frac{A}{N} \pm \frac{1}{2}\right)$$

$$SD = 1.620d\left(\frac{NB-A^2}{N^2} + 0.29\right), \frac{NB-A^2}{N^2} \geq 0.3$$

Or $SD = 0.53 \times d, \frac{NB-A^2}{N^2} < 0.3$  

$$A = \sum in_i$$

$$B = \sum i^2 \times n_i$$
\[ N = \sum n_i \]  

(6)

Only the failures or non-failures are used while calculating CFL and SD, depending on which have the smaller number of specimens. In formula (1), when the calculation was based on the non-failures, the minus (-) sign would be used, otherwise the plus sign (+) would be used. \( S_0 \) is the minimum stress level used in the measurement and \( d \) is the stress increment. The increment of 8 MPa was used in this study. The minimum stress level is denoted as \( i = 0 \) and the next stress level as \( i = 1 \), and so on. \( N_i \) is the number of failure or non-failure specimens at a given stress level.

**Antibacterial study**

In the thesis, Staphylococcus epidermidis Xen 43 was used to evaluate the antibacterial effect of the cements. Staphylococcus epidermidis Xen 43 is bioluminescent bacteria, whose number is proportional to the luminescence. The staphylococcus epidermidis Xen 43 was inoculated in tryptic soy broth (TSB) and kept at 37 °C for 18 h in the oven. The culture was then centrifuging at 2000 rpm to obtain a bacteria pellet. The concentration of bacteria was adjust to \( \text{OD}_{600} = 1.0 \) by re-suspending the pellet in PBS solution.

The direct contact test was used in the antibacterial measurement in which all the bacteria are allowed to stay on the samples and proliferate. Briefly, the prepared dental cements were placed in 96-well plates and 5 ml bacteria solution with \( \text{OD}_{600} = 1.0 \) was dispersed on the surface of the cements. The samples were then incubated at 37°C for 45 min, keeping the humidity inside the wells to avoid evaporation. 135 \( \mu \)L of TSB culture medium was added to each well after 45 min. A Hidex Chameleon plate reader was used to record the luminescence of the media every hour.

**The Gilmore needle method**

The setting time of the dental cements was determined by the Gilmore needle method. In this method, a needle with certain weight is placed on the surface of the cements. The cements are considered as set when there are no visible marks after placing the needle. The time from the start of mixing to no mark visible is defined as setting time. Two types of needles with different weights are used. A 113.4 g needle is used to determine the initial setting time while a 453.6 g needle is used for the final setting time.
ICP-AES

Inductively coupled plasma atomic emission spectroscopy (ICP-AES, Spetro Ciros CCD, Kleve, Germany) was used to measure the concentration of Ca$^{2+}$, Al$^{3+}$, Sr$^{2+}$, and Si$^{4+}$, measuring atomic Ca$^{2+}$ at 396.847 nm, Al$^{3+}$ at 167.078 nm, Sr$^{2+}$ at 407.771 nm, and Si$^{4+}$ at 251.612 nm. Ca$^{2+}$, Al$^{3+}$, Sr$^{2+}$, and Si$^{4+}$ in the extracts (diluted 100-fold with milliQ water) were measured in triplicate.
Sammanfattnings på svenska

Tändernas funktion och integritet kan förstöras av karies eller olyckor. Efter avlägsnande av karies eller läkning av skada, krävs dentala cement för att återställa den saknade tandstrukturken. De dentala cementen placeras i en hård oral miljö där de utsätts för varmechocker, konstant kemisk korrosion, och upprepad tuggkraft. Det ideala dentala cementet bör därför ha överlägsna mekaniska egenskaper, goda hanteringsegenskaper, biokompatibilitet, utmärkta antibakteriella egenskaper, och god bioaktivitet.

Glajonomercaement (GJC) är en av de mest använda dentala cementen nuförtiden. Konventionell GJC är baserad på reaktionen mellan polyakrylsyra (PAS) och glasmjöl som innehåller kiseldioxid, kalciumoxid, alumīnumoxid och fluorid. GJC anses överlägsen andra typer av dentala cement främst på grund av dess estetik och fluoravgivning under en längre tidsperiod. Nackdelarna med GJC inkluderar dess skörhet, känslighet för fukt, och ingen bioaktivitet på grund av utsläpp av oreagerad PAS. Denna avhandling presenterar vårt arbete med att utveckla dentala cement med förbättrade egenskaper. Det övergripande syftet var att öka bioaktiviteten och de antibakteriella egenskaperna hos dentala cement utan att påverka övriga egenskaper.


54
utvärderades. I vår studie, visade hartsmodifierade glasjonomercement och hartsbaserad komposit överlägsen statisk tryckhållfasthet och utmattningsgräns jämfört med konventionella glasjonomercement. Den statiska tryckhållfastheten av dentala cement ökade med åldringstiden. Åldring hade emellertid ingen effekt på den kompressiva utmattningsgränsen av hartsmodifierat glasjonomercement och hartsbaserad komposit. Tryckutmattningsgränsen för konventionell glasjonomercement visade även en drastisk minskning efter åldring.
Acknowledgements

So many people have supported and helped me during my four years’ journey in Uppsala and I would like to express my thanks to all of you.

To Håkan, my main supervisor, for providing me the opportunity to start the wonderful journey in Uppsala University and for giving me the freedom to explore in the lab. Your unique insights in the subjects are really impressive and I am always inspired by our discussions. To Wei, my co-supervisor, for your guidance on the experiments and your great patience. Your door is always open and I can find you whenever I need help. Special thanks to Gemma, my former officemate, for sharing your scientific experiences and your skills. I learned a lot from you on how to be a good researcher.

To Prof. Maria Strömme, for interviewing and recommending me to the MiM group. To Kristina, for providing accommodation when I first came to Uppsala. To Cristopher, for helping me so much during my first year in Sweden. I really cherish all the wonderful weekends we spent together in Örsundsbro.

I would like to thank all the members in the MiM group. To Caroline, for sharing your knowledge on micro-CT and fatigue measurement. To Cecilia, for helping me with statistical analysis issues. To Marjam, for involving me into the HA project. To Shiuli, for being a great collaborator and never give up spirits on the project. To Sara, for being so much fun in the lab and for giving me advices when I travelled to Italy and Spain. To Alejandro, for making the lab a better place to work. To Michael, for your optimism and great sense of humor. To Tao, for sharing the study and work information. To Torbjörn, for helping me revise the students’ lab reports. To Ingrid, for always helping me with the Swedish files. To Johan, for being a great officemate. I really enjoy the talks we have together. To Oscar, for the discussions on antibacterial test and for helping me with the Swedish summary. To Charlotte, Erik, Jun, Le, Dan, Xi, Thomas, Celine, Luimar, Lee, Susanne, Bang and other members in MiM group, thank you for all the great times. It has been great experiences to work with you together. Special thanks to our former group members, Carl, Johanna, Maryam, Maria, Bing for helping me with the equipments and measurements when I first came to the MiM group. To all the members in our division and in MST-group, I am encouraged by every greeting and warm smile.

Many thanks to my collaborators Kathryn, Shun, Satwik, Yixiao and Stefano. Thanks for helping me with my experiments. Special thanks to Prof.
Steven R. Jefferies, for the suggestions on the fatigue project and for the interesting discussions during the MRS conference. To Habtom, for helping me with the BET test. To Dr. Jean Pettersson, for your help with ICP measurement.

I would like to thank Hu Li, Jiangwei Liu, Wen Huang, Mingzi Jiao, Yurong Hu, Yi Ren, Xiaowen Li, Peng Zhang, Yan Guo, Da Zhang, Man Song, Xi Chen, Hongji Yan, Jinbao Zhang, Li Yang, Lei Zhang, Liyang Shi, Jingyi Hong, Yu Zhang, Ming Gao, Ruijun Pan, Dou Du, Hailiang Fang, Zhen Qiu, Yuxia Ji, Meiyuan Guo, Wenxing Yang, Shihuai Wang, Weijia Yang, Chenjuan Liu, Liguo Wang, Wei Li, Jiaojiao Yang, Kai Hua, Ling Xie, Jiangtao Chu, Zhen Liao, Changgang Xu, Xiao Yang, Miao Zhang, Shaohui Chen, Jinxing Huo, Yingying Zhu, Fengzhen Sun, Jiajie Yan, Changqing Ruan, Yuanyuan Han. I have had great time with you in Uppsala.

I would like to say thanks to Prof. Yin Liu and Prof. Linhua Jiang for guiding me to the research. Special thanks to Prof. Xuhua Ren for your encouragements and help.

Last but not the least, I would like to thank the support from my family. To my wife Shu, for your love and for all the things we have experienced together. Also to my parents-in-law for your support and consideration. To my parents, for your tireless dedication, for giving me a family full of love, for trusting and supporting my every choice. 谢谢爸爸妈妈，我爱你们！
References

42. Singh SP, Karmakar B. Mechanochemical synthesis of nano calcium silicate particles at room temperature. New journal of glass and ceramics 2011;01(02):49-52.
A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)