Microwave assisted calcium phosphate coating of biomedical implant materials

Anthony N. Passero

University of Toledo

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A Thesis

entitled

Microwave Assisted Calcium Phosphate Coating of Biomedical Implant Materials

by

Anthony N. Passero

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in

Mechanical Engineering

_________________________________________
Dr. Sarit Bhaduri, Committee Chair

_________________________________________
Dr. Hongyan Zhang, Committee Member

_________________________________________
Dr. Ioan Marinescu, Committee Member

_________________________________________
Dr. Patricia R. Komuniecki, Dean
College of Graduate Studies

The University of Toledo

May 2015
An Abstract of
Microwave Assisted Calcium Phosphate Coating of Biomedical Implant Materials

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Anthony N. Passero

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The University of Toledo

May 2015

This thesis investigates the feasibility of using a novel coating method to coat the biomedical implant materials polyether ether ketone (PEEK) and nitinol. In the field of orthopedics, many promising implant materials cannot easily bond directly to the surrounding bone. PEEK is a polymer which exhibits favorable mechanical properties and extreme chemical inertness. Nitinol is a nickel-titanium shape memory intermetallic which can undergo deformation when heated. This allows fixation of surrounding bone and tissues. While neither material is suitable to use as is, a coating of calcium phosphate (the primary mineral component of bone) has been shown to promote bone attachment. Many methods exist for depositing this coating, but the biomimetic method is attractive for its simplicity and high degree of similarity to actual bone chemistry. A faster formulation of the biomimetic method using microwave irradiation was used to coat PEEK and nitinol substrates. This coating was examined using several characterization methods, including scanning electron microscopy, energy dispersive spectroscopy, X-ray diffraction, and water contact angle measurements. The coatings exhibited the desired properties necessary for bone attachment, and warrant further clinical study.
Acknowledgements

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Last and most importantly, I would like to thank my friends, my family, and my wife Mrs. Elizabeth Passero. I cannot imagine undertaking this without her support, and I am truly grateful.
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List of Abbreviations

Ca-P..................................Calcium Phosphate
EDS..................................Energy Dispersive Spectroscopy
HA....................................Hydroxyapatite
PEEK..................................Polyether Ether Ketone
SEM .................................Scanning Electron Microscopy
XRD .................................X-ray Diffraction
Chapter 1

Introduction

Optimizing the performance of the bone-interface of biomedical implants has become the subject of significant research, spurred on by the need for bone replacements. Currently, more than 2 million fractures result from osteoporosis in the United States alone, and cost more than $19 billion each year [1]. Typically, bone substitution procedures utilize materials from the patient (autografts), or failing this, natural substitutes (allografts). This eliminates the possibility of immune-based inflammation, which can lead to failure of the procedure. Unfortunately, this is not always possible, and thus the only alternative is to utilize artificial implants with favorable biological responses. Coating the implant surface with appropriate materials increases the bioactivity of such an implant, enhancing osseointegration (strong bone-interface).

Although many implant materials are biocompatible and cause no inflammatory response, the body may recognize the implant as a foreign object and surround it with fibrous tissues in order to isolate it [2]. This results in a nonmineralized layer between the implant and surrounding bone, which may be insufficient to rigidly constrain the implant [3]. It was found by Branemark et al. that careful surgical procedures can minimize the thickness of this fibrous layer, which is essentially made of scar tissue [4]. When this is
achieved, the implant surface attaches directly to the surrounding bone with no intervening tissues, leading to osseointegration. To anchor the implant even further, true chemical bonding of bone to implant must be achieved by optimizing the surface properties of the implant.

Direct bonding with bone can be achieved in the presence of a bioactive calcium phosphate (Ca-P) coating on the implant surface. A coating is preferred over an implant made entirely of Ca-P because the bulk mechanical properties of such materials are often insufficient for typical loadings. Hydroxyapatite (HA), Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, is the Ca-P commonly chosen for this purpose because it is the primary mineral component in bone [1,5-10]. A relatively thin layer of HA on an implant such as titanium thus provides favorable surface conditions for biocompatibility between the implant and surrounding tissues without sacrificing the excellent mechanical properties of titanium implants. Many methods are currently available to provide this Ca-P coating.

Coating procedures may make use of either a physical or chemical process. Physical coating methods include Plasma Spraying [11,12], Dip Coating [13-15], Sputtering [16-20], Electrophoretic Deposition [21-23] and Pulsed Laser Ablation [24,25]. These and several other methods are shown in Table 1.1. Though very common in industry, nearly all physical methods are line of sight processes, which can only be used to coat implants of the simplest geometries. Most processes also utilize high temperatures, which have been seen to cause thermal degradation of the coatings [2]. This can cause insufficient adhesion to the substrate as well as excessive dissolution, both of which may lead to premature failure of the implant and the formation of particulate debris. The generation of high temperatures may also prevent the incorporation of
Table 1.1: Various Ca-P coating techniques [26].

<table>
<thead>
<tr>
<th>Technique</th>
<th>Thickness</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal spraying</td>
<td>30 to 200 µm</td>
<td>High deposition rates; low cost</td>
<td>Line of sight technique; high temperatures induce decomposition; rapid cooling produces amorphous coatings; high temperatures prevent from simultaneous incorporation of biological agents</td>
</tr>
<tr>
<td>Plasma spraying</td>
<td>30 to 200 µm</td>
<td>High deposition rates; improved wear and corrosion resistance and biocompatibility</td>
<td>Line of sight technique; high temperatures induce decomposition; rapid cooling produces amorphous coatings; high temperatures prevent from simultaneous incorporation of biological agents</td>
</tr>
<tr>
<td>Magnetron sputtering</td>
<td>0.5 to 3 µm</td>
<td>Uniform coating thickness on flat substrates; high purity and high adhesion; dense pore-free coatings; excellent coverage of steps and small features; ability to coat heat-sensitive substrates</td>
<td>Line of sight technique; expensive; low deposition rates; produces amorphous coatings; high temperatures prevent from simultaneous incorporation of biological agents</td>
</tr>
<tr>
<td>Pulsed laser deposition</td>
<td>0.05 to 5 µm</td>
<td>Coatings with crystalline and amorphous phases; dense and porous coatings; high adhesive strength</td>
<td>Line of sight technique; expensive; high temperatures prevent from simultaneous incorporation of biological agents</td>
</tr>
<tr>
<td>Ion beam deposition</td>
<td>0.05 to 1 µm</td>
<td>Uniform coating thickness; high adhesive strength</td>
<td>Line of sight technique; expensive; produces amorphous coatings</td>
</tr>
<tr>
<td>Dynamic mixing method</td>
<td>0.05 to 1.3 µm</td>
<td>High adhesive strength</td>
<td>Line of sight technique; expensive; produces amorphous coatings</td>
</tr>
<tr>
<td>Dip and spin coating</td>
<td>2 µm to 0.5 mm</td>
<td>Inexpensive; coatings applied quickly; can coat complex substrates</td>
<td>Requires high sintering temperatures; thermal expansion mismatch</td>
</tr>
<tr>
<td>Sol-gel technique</td>
<td>&lt; 1 µm</td>
<td>Can coat complex shapes; low processing temperatures; thin coatings; inexpensive process; can incorporate biological molecules</td>
<td>Some processes require controlled atmosphere processing; expensive raw materials</td>
</tr>
<tr>
<td>Electrophoretic deposition</td>
<td>0.1 to 2.0 mm</td>
<td>Uniform coating thickness; rapid deposition rates; can coat complex substrates; can incorporate biological molecules</td>
<td>Difficult to produce crack-free coatings; require high sintering temperatures</td>
</tr>
<tr>
<td>Electrochemical (cathodic)</td>
<td>0.05 to 0.5 mm</td>
<td>Good shape conformity; room temperature process; uniform coating thickness; short processing times; can incorporate biological molecules</td>
<td>Sometimes stressed coatings are produced, leading to their poor adhesion with substrate; requires good control of electrolyte parameters</td>
</tr>
<tr>
<td>Biomimetic process</td>
<td>&lt; 30 µm</td>
<td>Low processing temperatures; can form bonelike apatite; can coat complex shapes; can incorporate biological molecules</td>
<td>Time consuming; requires replenishment and a pH constancy of the simulating solutions</td>
</tr>
<tr>
<td>Hot isostatic pressing</td>
<td>0.2 to 2.0 µm</td>
<td>Produces dense coatings</td>
<td>Cannot coat complex substrates; high temperature required; thermal expansion mismatch; elastic property differences; expensive; removal/interaction of encapsulation material; high temperatures prevent from simultaneous incorporation of biological agents</td>
</tr>
<tr>
<td>Micro-arc oxidation</td>
<td>3 to 20 µm</td>
<td>Simple, economical and environmentally friendly coating technique; suitable for coating of complex geometries</td>
<td>Except of calcium orthophosphates, coatings always contain admixture phases</td>
</tr>
</tbody>
</table>

biological agents into the coating, such as in drug delivery. Furthermore, many substrate materials with lower melting points simply cannot be used in the presence of such high temperatures, due to thermal degradation of the substrate itself. While somewhat limited
in efficacy by these shortcomings, physical coating processes are quite common and can be useful in appropriate situations.

Of the processes listed in Table 1.1, plasma spraying is the most commonly utilized coating process in industrial settings. This process is illustrated in Figure 1-1. Commercially available plasma spraying equipment first became available as early as the 1960s, supplemented by the technology accompanying the famous “space race” [2]. This equipment was most commonly used in the aerospace industry, with subsequent research into its potential application in the biomedical field. The first patent for a plasma-sprayed HA coating technique appeared in Japan in 1975, and since then the technology has evolved and seen widespread application [27].

**Figure 1-1:** Common configuration of the plasma spraying coating methodology for producing HA coatings [28].
The mechanism of plasma spraying first involves the creation of a plasma jet by ionizing an inert gas (often argon). A powdered HA feed is provided to the system, which is then melted by the superheated plasma and projected toward the substrate surface. While this process is able to quickly deposit a very high volume of material, it suffers from numerous drawbacks common to physical deposition processes, as noted in Table 1.1 [26]. These problems include line of sight deposition and issues related to the extremely high temperatures used, such as delamination, decomposition, and the inability to incorporate biological agents. As the core of the plasma jet can reach temperatures as high as 12,000 K, these temperature-related problems are unavoidable and inherent to the methodology itself. Nevertheless, plasma spraying provides an efficient means of quickly producing coatings in certain situations.

Dip coating is another physical coating technique that is of significance. This coating methodology entails submerging the implant into a sol containing Ca-P [29]. The samples are then removed at a closely controlled rate, which is a key factor in determining the coating thickness. This is followed by a heating step used to evaporate all remaining solvent and further densify the coating. Coating thickness can be increased to an arbitrary desired thickness by applying subsequent dip treatments over the previously-formed coatings [13]. The underlying parameter behind this process that allows coating in this manner is the viscosity of the coating fluid. This, along with the speed at which the substrates are removed, controls the coating thickness. This process is illustrated in Figure 1-2. There seems to be some confusion in the literature about the terminology for dip coating. Perhaps because of its similarity to the biomimetic route of coating (submersion of substrates into a coating solution), some biomimetic studies have been
Figure 1-2: General configuration illustrating the dip coating methodology [30].

labeled as “dip coating” [31]. However, these two coating processes rely on very
different underlying processes and should not be used interchangeably. In dip coating,
previously-formed HA in solution attaches to sample surfaces through physical, rather
than chemical, means. On the other hand, biomimetic coating involves a HA layer being
“built up” ion by ion on the surface of the implant, and is thus categorized as a chemical
method.

When speaking of dip coating, it is important to mention spin coating as well.
Spin coating utilizes a similar underlying mechanism, also employing a solution with
dissolved HA powder. However, instead of submerging substrates into this solution, the
sample (typically a flat disk) is spun at a specific speed and the coating solution is poured
onto it. The force of rotation directs the fluid radially outward, with rotation speed and fluid viscosity determining the resulting thickness [15]. Controlling this rotation speed is analogous to controlling the removal speed in dip coating. Because dip coating and spin coating share the same underlying mechanism and are similar in operation, they are on occasion used interchangeably to refer to one another. While dip/spin coating is quite simple and inexpensive, it is similar to plasma spraying in that it entails excessively high temperatures during the heating step following the dipping [14, 15]. These temperatures are encountered after the coating solution has covered the substrate surface, and are necessary to evaporate the solvent and densify the final coating. As with plasma spraying, this can cause several problems, such as delamination, decomposition, and the inability to incorporate biological molecules.

Sputter coating offers unique advantages in certain areas as compared to other physical methods, yet still exhibits common problems. The coating process involves the bombardment of a target with plasma under vacuum conditions. This target is a compressed powder composed of the coating material [20]. An inert gas, such as argon, is ionized using a large electric potential difference, and is accelerated to strike this target. Such a collision scatters atoms of the target throughout the chamber, some of which come into contact with the substrate and adhere to it. The general layout of the process is shown in Figure 1-3. It is somewhat unusual among physical coating methods in that even though it involves high temperatures because of the plasma, these temperatures are not in direct contact with the substrate. The plasma only contacts the HA target, and although this prevents incorporation of biological agents, substrates with lower melting temperatures, such as polymers, can be used [32]. While this demonstrates an advantage
over thermal spraying methods, deposition rates are low and the sputtering targets of proper chemistry are expensive. Additionally, sputter coating is a line of sight process, and cannot be used to coat complex geometries. Thus, while sputter coating of HA remains useful for certain research applications, it is not suitable for widespread commercial application.

Electrophoretic deposition provides another physical route for producing uniform coatings on a variety of substrates, including complex substrates. A solution containing dissolved HA is necessary, similar to that found in dip/spin coating. Two electrodes are placed in the water, and one is connected to the substrate. This creates an electric potential gradient in the coating solution, which draws particles to the substrate. This
process is illustrated in Figure 1-4. This method is therefore not line of sight, and any portion of the substrate that is exposed to the solution can be coated, allowing coating of complex surfaces [23]. The resulting coating is also quite uniform, owing primarily to the homogeneous nature of electrical field distribution near the surface of conductors. This is typically followed by a high temperature sintering step, to further densify the coatings. Because of this step, many problems common to the physical coating methods are unfortunately present in electrophoretic coating as well, including the inability to coat polymers with low melting temperatures. The primary means of controlling the deposition process is variation of the electric potential between the electrodes, as well as the particulars of the coating solution (composition, pH, etc.) [21,22]. Careful control of these parameters allows the deposition of uniform coatings with relatively high purity, but still suffer from high temperature complications and limitations.
Pulsed laser ablation is a coating method quite similar to sputtering, though different in some significant ways. As in sputtering, materials are stripped off a target, usually a flat disc, and travels through a vacuum chamber to nucleate on the substrate. However, while sputtering distributes the target material throughout the chamber due to the simple momentum of the collisions of gas ions with the target, the process in laser ablation is far more complex. Each laser pulse vaporizes a portion of the surface, and the intense electromagnetic fields of the laser quickly ionize the vaporized target atoms, forming a plasma jet [36]. The laser ablation process is illustrated in Figure 1-5. Electrons freed by this are accelerated by these electromagnetic fields and impact the target, releasing more material. Control of gas pressure during deposition is essential, as once deposition has begun the plasma jet created by the laser can sputter a portion of the
formed coating if the pressure is too low. This process is often referred to as “back-sputtering.” Alternately, if the pressure is too high, transfer of the particles to the substrate is hindered. However, fine tuning of pressure allows a greater rate of deposition than back-sputtering removal, and net growth occurs [37]. Pulsed laser ablation is a flexible process in that many different morphologies of coatings can be produced, including crystalline and amorphous structures [24]. Additionally, it shares the attribute of superior adhesion with the sputtering process, due to the similar nucleation conditions. However, it is also quite expensive, is a line of sight process, and due to the high energy of the laser and formation of a plasma jet, very high temperatures are involved, and prohibit incorporation of biological molecules [24,25]. Due to these problems, pulsed laser ablation while useful in some capacities suffers from many shortcomings common to physical processes, and new coating approaches are needed.

It appears that all physical coating processes employ either a line of sight deposition or involve high temperatures, and many feature both. To combat these issues, chemical processes to produce HA coatings such as sol-gel [38-41] and biomimetic coating [42-46] are used. These are often preceded by a separate chemical treatment to etch the surface and create appropriate functional groups which induce apatite formation on the surface. These treatments consist of either an acid [47,48], hydrogen peroxide [49-52], anodic oxidation [53-55], or a strong base [56-58], and can even be used on their own to improve bioactivity of the implant without a Ca-P coating.

Sol-gel deposition is a widely used chemical process for producing high quality Ca-P coatings. Substrates surfaces are covered with a solution which evolves into a colloidal gel. This solution usually contains chemicals which combine in solution to
create HA (such as triethyl phosphate and calcium nitrate), which constitutes the solid phase of the gel [40, 41], but may also contain a powdered form of HA [38]. The solution can be applied to substrates using principles of dip [40] or spin [41] coating. The sol-gel process is illustrated in Figure 1-6. To form a coating from this configuration, the liquid phase must be removed. This is usually accomplished through applying heat (often at 70-80°C) and allowing the substrates to dry, leaving behind the solid phase of HA. Following this, annealing is typically performed at temperatures up to 600°C to further densify the coating [38, 40, 41]. While this temperature range is quite low when compared to many physical methods, it is still high enough to preclude the use of several polymer implant materials. Additionally, the sol-gel process utilizes costly materials, and
similar to sputtering deposition, may not always be economical. Nevertheless, sol-gel deposition offers distinct advantages over many competing processes, such as lower temperatures and the ability to coat complex substrates.

Many of the preceding coating methodologies offer unique advantages over others and may be of scientific and, in some cases, industrial interest. However, each process has associated problems that can be difficult to overcome, depending upon the specifics of the application. All processes, excepting sputtering, involve temperatures too high to permit many common polymer substrates, such as polyetheretherketone (PEEK), to be used as implant substrates. Some of these same processes also involve even higher temperatures which, in addition to preventing low melting point materials from being used, also preclude biological molecules for controlled drug release from being used. Sputtering, however, is not exempt from problems, as it and many other physical processes are line of sight, and cannot coat complex surfaces. Because biomedical implants may take on quite complicated shapes, the ability to coat complex surfaces is highly sought after. Finally, several processes mentioned previously, including sol-gel deposition and sputtering, either involve expensive materials or require expensive processing, and for this reason may not be economically viable for industrial application. Thus, a need exists for an inexpensive method capable of coating complex substrates at low temperatures. Biomimetic coating provides such a method.

The biomimetic methodology, as the name suggests, seeks to mimic human physiological conditions to maximize biocompatibility. While replicating every feature of human physiology down to the cellular level is obviously impossible, selection of key parameters yields a reasonable approximation. Reproducing the ionic concentrations of
human blood plasma, holding the solution at a neutral pH of 7.4, and fixing the temperature at human body temperature (37°C) provides sufficient conditions for bonelike HA to form. The coating solution was first introduced by Kokubo et al. and contained ionic concentrations similar, but not identical, to human blood plasma [60]. These coating solutions are typically formulated so that HA does not spontaneously form homogenously in solution, which would wastefully deplete the ions needed for coating. Instead, the solution is constructed to be stable under physiological conditions, but able to precipitate in very close proximity to the implant surface. Functional groups created by either alkali treatment or glass particles, as in the original experiment of Kokubo et al., lower the solubility of HA in the immediate vicinity of the implant surface, allowing coating formation [61].

Among the chemical methods, only the biomimetic route offers the attractive feature of “bonelike” HA [62]. In natural bone, several ionic substitutions are made in the Ca_{10}(PO_4)_{6}(OH)_2 structure of HA, often by the carbonate ion, although other substitutions by magnesium and hydroxide ions can occur [63,64]. Biomimetic coating methods are able to create HA that contains these substitutions, resulting in bonelike coatings. These bonelike coatings have been found to exhibit superior biocompatibility with osteoblast cells when compared to other common methods [46]. Biomimetic coating also features the advantages of low cost and the ability to coat complex substrates, with the only drawbacks being related to the extensive coating time required. Thus, coating via biomimetic methods shows great promise for the future.

Because the coating solution was designed to emulate human body conditions, it has been termed “simulated body fluid” (SBF). Since its initial formulation, many
**Table 1.2**: Ion concentrations found in several proposed SBF solutions compared with those found in human blood plasma [65,66].

<table>
<thead>
<tr>
<th>Ion</th>
<th>Blood</th>
<th>c-SBF</th>
<th>r-SBF</th>
<th>i-SBF</th>
<th>m-SBF</th>
<th>t-SBF</th>
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<tr>
<td>Na⁺</td>
<td>142.0</td>
<td>142.0</td>
<td>142.0</td>
<td>142.0</td>
<td>142.0</td>
<td>142.0</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>103.0</td>
<td>147.8</td>
<td>103.0</td>
<td>103.0</td>
<td>103.0</td>
<td>125.0</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>27.0</td>
<td>4.2</td>
<td>27.0</td>
<td>27.0</td>
<td>10.0</td>
<td>27.0</td>
</tr>
<tr>
<td>K⁺</td>
<td>5.0</td>
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<td>5.0</td>
<td>5.0</td>
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</tr>
<tr>
<td>Mg²⁺</td>
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<td>1.5</td>
<td>1.5</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
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<tr>
<td>Ca²⁺</td>
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<td>HPO₄²⁻</td>
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<tr>
<td>SO₄²⁻</td>
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</tbody>
</table>

Alternative solutions have been proposed to replace the original SBF (c-SBF), some again by Kokubo (r-SBF, i-SBF, and m-SBF) [65] and others (t-SBF) [66]. The ionic concentrations of these solutions, as well as those of human blood plasma, are shown in Table 1.2. While all SBF solutions approximate ionic concentrations of blood plasma to a certain degree, stability is an issue for both r-SBF and i-SBF. These were found to display significant stability problems after 4 weeks storage at 5°C or 2 weeks storage at 36.5°C [65]. Additionally, Tas developed an SBF solution which more closely models the Cl⁻ and HCO₃⁻ content of blood plasma without sacrificing stability [66]. Higher HCO₃⁻
content is very important, as bonelike HA contains carbonate substitutions, and therefore at the present time t-SBF is most attractive for biomimetic coatings.

The coating procedure itself typically consists of immersing alkali pre-treated substrates in SBF until a coating of sufficient thickness has been formed [45]. Alkali pre-treatment forms a hydrous layer on the substrate. For the case of a titanium substrate, sodium (or potassium) titanate is formed, shown in Figure 1-7 [4]. This layer is necessary for coating, as soaking in SBF causes the cations in the layer to exchange with \( \text{H}_3\text{O}^+ \) ions in the solution, forming hydroxide groups on the surface of the substrate [67]. Originally, instead of an alkali pre-treatment, Kokubo et al. utilized glass particles placed on the substrate surface as the cation source, as illustrated in Figure 1-8, though this method has largely been replaced with alkali pre-treatment [60]. These hydroxide groups increase the ionic activity of HA in the localized region around the substrate, allowing coating nucleation without general precipitation and associated loss of ions from solution. This
nucleation is initially amorphous, with particles on the order of nanometers, and can grow to form larger, crystalline structures after sufficient soaking time in SBF [68].

Biomimetic coating, when compared to more widespread coating methodologies such as plasma spraying, is relatively new. The few biomimetic studies that have been performed have indicated promising results. Barrere et al. observed that after implantation into rats, carbonated apatite coated on titanium calcified into the surrounding bone and showed no signs of toxicity [69]. This coating exhibited superior behavior when compared to a biomimetic coating which was not carbonated. The unique ability of the biomimetic approach to form bonelike, carbonated apatite thus enables better implant behavior. Kokubo et al. also conducted a biomimetic in vivo study in both rats and rabbits using the polymer polyethersulphone (PES) as a substrate [61]. All
coatings investigated degraded at an acceptable rate for bone growth to extend up to the bare PES surface with no failures in fixation. Furthermore, by varying the SBF composition, the crystallinity of the coating can be controlled, and it was seen that this affects the degradation rate, with amorphous coatings degrading faster than highly crystalline coatings. Thus, the biomimetic method enables a simple means of adjusting composition and thereby coating degradation rate to a suitable level for a given application. Biomimetic results in vivo have shown excellent biocompatibility and offer unique benefits that other approaches do not, indicating great potential for further application.

Although biomimetic coating offers many advantages over alternative methods, it is time consuming, and recent work has focused on constructing timescales feasible for industrial application. In its original implementation, the biomimetic method used by Kokubo et al. required approximately 14 days to completely coat the substrate, which is far too long to be appropriate for industry [60]. In order to decrease the time required for coating, efforts have been made to increase the concentration of ions in the SBF. The ionic concentration of each constituent in the SBF can be increased by a factor of five or even ten, and the time required for coating has drastically been reduced [45, 70]. Table 1.3 shows the required soaking time for various concentrations of SBF. In this manner, it has been possible to produce a 22 µm thick coating in as little as two hours. One difficulty associated with increasing SBF concentration is the degree of supersaturation, as SBF is supersaturated when operating at physiological pH [71]. This can be overcome by using CO₂ bubbling or other buffering agents to adjust the pH to values lower than the physiological 7.4 when using concentrated forms. Thus, a departure from strictly
Table 1.3: Required soaking time to produce a biomimetic coating for various SBF concentrations [45, 60, 72-75].

<table>
<thead>
<tr>
<th>SBF Concentration</th>
<th>Required Soaking Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>14-20 days</td>
</tr>
<tr>
<td>4</td>
<td>7 days</td>
</tr>
<tr>
<td>5</td>
<td>1-2 days</td>
</tr>
<tr>
<td>10</td>
<td>2 hours</td>
</tr>
</tbody>
</table>

physiological conditions such as pH and ionic concentrations allows for a similar but more practical coating approach.

Work done recently by Bhaduri et al. has modified the biomimetic process by introducing microwave irradiation, drastically reducing coating times [76]. It was previously found that in the presence of microwave radiation, amorphous Ca-P nuclei will form spontaneously in SBF [77, 78]. These amorphous precursors are similar to those found in the first stages of biomimetic coating. In this coating methodology, amorphous precursors form throughout the SBF, and those that form on the surface of the sample can act as nuclei for further crystal growth. The formation of a thin, amorphous Ca-P coating in this manner is illustrated in Figure 1-9. Because the entire microwave process takes only a few minutes to complete, it is possible to produce HA coated substrates extremely quickly, compared to other biomimetic methods. In this way, an extremely thin HA layer may be formed consisting entirely of amorphous nuclei, or these nuclei can be used to grow crystals in SBF to any thickness desired. The as-formed amorphous coating was seen to exhibit favorable biological properties via
Figure 1-9: Ca-P formation for conventional biomimetic coating and microwave assisted biomimetic coating [76].

cytocompatibility tests [76]. Originally performed on titanium substrates, one of the primary focuses of this work was to investigate the viability of the method for alternative substrates, such as PEEK and a porous Ti-Ni alloy.

To meet the growing need for biomedical implants, biomimetic coating offers several key advantages over comparable methods, and shows great promise for the future. Its philosophy of emulating natural processes honed over countless years by evolution produces the only coating methodology capable of forming bonelike apatite, by means of the SBF solution. This variety of apatite has been seen to exhibit superior biocompatibility, and can be coated onto complex surface geometries and incorporate biological molecules for applications such as drug delivery. Furthermore, all materials
and equipment are very inexpensive, and the only drawback of the entire process is the lengthy timeframe required for its completion. However, new advances in concentrated SBF solutions have reduced this to hours, and microwave assisted studies have drastically reduced even this to mere minutes. The subject of this work is to engineer this microwave assisted process for other materials such as PEEK and a porous TiNi alloy. The eventual outcome of this work may at last make the biomimetic process commercially viable for the orthopedic industry.
Chapter 2

PEEK

2.1 Introduction

Calcium phosphate (Ca-P) coatings are often applied to biomedical implant surfaces in order to allow direct bonding with bone [64]. Known as osseointegration, this direct bond with no intervening soft tissues allows rigid mechanical fixation, forestalling failure under loading [3]. While many methods of producing this coating exist, most have serious drawbacks related to both extremely high temperatures and high cost. The biomimetic approach overcomes these obstacles and also offers the unique advantage of being able to produce a bone-like coating: hydroxyapatite (HA), \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \), which contains various ionic substitutions such as the carbonate ion [67,79]. To achieve this, an alkali pre-treatment involves submerging substrates, usually titanium, into an acid, base, or hydrogen peroxide [51,80]. Cations from the particular alkali treatment used (for example, \( \text{Na}^+ \) in \( \text{NaOH} \)) form a titanate layer on the surface of the implant [4]. When subsequently immersed in a simulated body fluid (SBF) this layer causes the formation of amorphous Ca-P precursors in the immediate vicinity of the implant. These grow into large crystals as \( \text{Ca}^{2+} \) and \( \text{PO}_4^{3-} \) ions are consumed from the SBF solution over time [81].
The biomimetic coating methodology is thus an attractive means of altering the surface of an implant to enhance osseointegration.

While biomimetic coating offers several unique advantages, it is a very slow process in its original form. Recent advances have been able to reduce this timescale from weeks to hours by increasing the solution concentration [45,60]. While this is a considerable improvement, even shorter times are required for widespread use in industry. To this end, Bhaduri et al. have investigated the effect of microwave irradiation on SBF [77]. It was found that amorphous calcium phosphate (ACP) nanosphere precursors were formed homogeneously in solution, without further crystal growth. This precipitation is thought to occur because in SBF under microwave irradiation, ACP is first to precipitate, followed sequentially by octacalcium phosphate and HA, as stated by Lerner et al [82]. A bioactive substrate submerged in solution in such a situation will form ACP precursors completely covering the surface, as performed by Bhaduri et al. on Ti6Al4V substrates [76]. This microwave-assisted biomimetic coating methodology allows complete coverage of the substrate by a thin ACP layer in mere minutes.

Polyether ether ketone (PEEK) biomedical implants are a growing class of implant materials which provide advantages over more traditional materials, such as titanium. PEEK is a highly inert polymer which was originally utilized in aircraft and turbine blades due to its strength and high resistance to thermal and chemical degradation [83]. Following this, it began to be considered for biomedical implant applications in the late 1980s, for many of the same reasons [84]. In particular, controlling the polymer fabrication process allows for the adjustment of the elastic modulus to a desired result. Titanium, the most commonly used implant material, has an elastic modulus as high as
ten times that of human bone [85]. Because of this, the bone surrounding the titanium implant experiences a much lower stress, while the implant experiences a high stress state; this phenomenon is known as “stress shielding”. This situation is deleterious to the bone tissue, which is accustomed to a certain level of stress and in the absence of it will weaken, causing injury [86]. By adjusting the elastic modulus of PEEK to a value approximately equal to that of bone, this stress shielding can be virtually eliminated. Additionally, the chemical inertness of PEEK allows acceptance of the implant by the body, causing no toxicity and minimal inflammation [83,84]. For these reasons, PEEK is an excellent candidate for study as a biomedical implant material.

The present work aims to investigate the viability of the microwave-assisted coating methodology for PEEK substrates. This material is extremely inert, and as such it does not display toxicity, but unfortunately also results in no bioactivity. Thus, alkali treatment allows for the necessary functional groups to form on the implant surface. However, because PEEK is so inert, the etching process must be intensified and this work employs microwave-assisted NaOH etching to this end. PEEK substrates were coated using microwave-assisted biomimetic deposition, forming an ultra-thin ACP layer covering the implant surface. If a thicker coating is desired, the as-deposited ACP layer may be grown in concentrated SBF to the desired thickness, following more conventional approaches. In this manner the production of bioactive calcium phosphate coatings on PEEK for biomedical implant application is investigated.
2.2 Experimental Procedure

2.2.1 Material Preparation

Cylindrical PEEK rods with a 3/16 inch diameter were purchased from Precision Punch & Plastics. These rods were cut into 1/10 inch disks in the Mechanical, Industrial, and Manufacturing Engineering Department at the University of Toledo. The samples were cleaned ultrasonically in acetone, 70% ethanol, and deionized water for 10 minutes each, subsequently. Following this, samples were air-dried overnight. All chemicals were purchased from Fisher Scientific.

An intense alkali pre-treatment is required to form the functional groups necessary for apatite growth for a material as inert as PEEK. Pino et al. utilized a 48 hour 10 M NaOH treatment to this end, which was also adopted in this study [87,88]. This pre-treatment was carried out in a tightly capped bottle stored at approximately 60°C. To further intensify the etching, a NaOH microwave etching processes was utilized. This involved subjecting the substrates, immersed in 10 M NaOH solution, to microwave irradiation for a total of five minutes, on 60% maximum power in a 1200 W microwave oven (Panasonic). During this process, it was necessary to exchange the samples and solution between two different glass bottles approximately every 40 seconds, due to extensive cracking of the glass from overheated evaporated NaOH. Because PEEK is buoyant in 10 M NaOH, a thin piece of cheesecloth (HDX – Home Depot) was loosely wrapped around the substrates and weighted down with a small piece of inert alumina ceramic for both phases of the etching process. To maximize etching time, samples were
2.2.2 Microwave Assisted Coating

The coating solution is an SBF with ionic concentrations made to imitate human blood plasma. However, in the interest of further optimizing coating processes, certain concentrations have been modified in ways slightly deviating from physiological conditions. To prepare the coating solution, the following chemicals were dissolved in approximately 950mL of water in the following order: 1.135g KCl, 27.986g NaCl, 0.907g NaHCO$_3$, 0.163g MgCl$_2$$\cdot$6H$_2$O, 1.47g CaCl$_2$$\cdot$2H$_2$O, 0.114g Na$_2$SO$_4$, and 0.545g K$_2$HPO$_4$. The ionic concentration of each constituent of the SBF solution is shown in Table 2.1. As can be seen, the solution represents a somewhat of a departure from biological concentrations. This allows the coating process to be altered to facilitate desirable coating formation while still utilizing the biomimetic process. To allow sufficient time for dissolution, three minutes were elapsed for stirring prior to adding the next chemical. This solution can be stored in a tightly capped bottle at room temperature for several weeks without precipitation.

Immediately prior to coating, PEEK substrates were removed from the 10 M NaOH solution and rinsed gently with DI water using a pipette. These were submerged into 100 mL of SBF solution contained in a glass bottle. A piece of inert alumina ceramic was placed over the opening of this bottle. This covered most of the area of the opening, leaving a small portion of the opening exposed, which prevented pressure buildup within the bottle while at the same time minimizing loss of heat and coating solution during boiling. These were placed near the center of the 1200 W microwave oven and irradiated
Table 2.1: Ion concentrations of constituents of PEEK coating solution compared to those of human blood plasma. [66]

<table>
<thead>
<tr>
<th>Ion</th>
<th>Human Blood Plasma</th>
<th>PEEK Coating Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)</td>
<td>142.0</td>
<td>490.9</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>103.0</td>
<td>515.3</td>
</tr>
<tr>
<td>HCO(_3)(^-)</td>
<td>27.0</td>
<td>10.8</td>
</tr>
<tr>
<td>K(^+)</td>
<td>5.0</td>
<td>21.5</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>2.5</td>
<td>10.0</td>
</tr>
<tr>
<td>HPO(_4)(^{2-})</td>
<td>1.0</td>
<td>3.1</td>
</tr>
<tr>
<td>SO(_4)(^{2-})</td>
<td>0.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

at full power for four minutes. Following this, the configuration was allowed to cool in the microwave for one minute, before transferring the samples to another glass bottle containing 100 mL of SBF solution. In this way, the samples were subjected to another microwave coating procedure. This was done to ensure uniform coverage of the substrate surface, as only surfaces exposed to the solution can nucleate the ACP precursors necessary for coating formation. Following this second coating treatment, the PEEK samples were gently rinsed with DI water and allowed to dry overnight in a furnace at 60°C.
2.2.3 Characterization

Hydrophilicity was assessed before and after coating of PEEK using a water contact angle meter (Model CAM-MTCRO, Tantec), and subjected to a one-way ANOVA analysis (n=4). Samples were examined using X-ray diffraction (XRD, Ultima III, Rigaku) using monochromated Cu Kα radiation operated at a 40 kV voltage and 44 mA current. This was performed in parallel beam mode, due to the very thin SBF coating. Visual features were investigated using scanning electron microscopy (SEM, S4800, Hitachi) micrographs. Because moderate conductivity of samples is required for successful use of SEM, the PEEK surface was further altered for visual clarity to this end. This was achieved using a gold sputter coating (Cressington Sputter Coater 108 Auto) for a duration of 30 seconds. With this treatment, samples were made sufficiently conductive for successful SEM characterization. Elemental composition of the coatings was made possible using the energy dispersive spectroscopy (EDS) attachment of the SEM. These methods allowed a comprehensive examination of the coating formed by microwave assisted biomimetic methods.

2.3 Results and Discussion

After both the etching and coating processes, the appearance of PEEK substrates to the naked eye remains unchanged, as seen in Figure 2-1. This could potentially cause problems if, before implantation, healthcare professionals wished to confirm the presence of a coating. However, this problem can be quickly and inexpensively solved when considering the extreme difference in hydrophilicity of coated and uncoated PEEK.
Figure 2-1: Visual comparison of uncoated (left), MW etched (center), and MW coated (right) PEEK substrates.

Hydrophilicity is easily indicated by the water contact angle, with a lower angle corresponding to a more hydrophilic surface. Water contact angle results can be seen in Figure 2-2. Uncoated PEEK was found to have a water contact angle of $81 \pm 4.1^\circ$, the highest observed by a large margin. To illustrate the efficacy of the MW etching process, the water contact angle was found to be $51.5 \pm 4.4^\circ$ for etched PEEK. After a microwave assisted biomimetic coating procedure, this contact angle was drastically reduced to $8.7 \pm 4.2^\circ$. Thus, hydrophilicity was found be very significantly affected by etching and coating processes ($p < 0.001$).

It should be noted that while testing the coated sample, the water droplet deposited on the surface spread over the entire top surface of the PEEK. This implies that, given a larger surface area, an even lower contact angle would be observed, and therefore even higher hydrophilicity is likely present in the coating. However, the configuration of the water contact angle meter does not allow for a larger top surface, so such a test cannot be done. However, the results obtained for coated PEEK can still be
Figure 2-2: Water contact angle measurements conducted for PEEK with no prior treatment (Uncoated), microwave assisted etching (MW Etched), and microwave assisted biomimetic coating (MW Coated).

used as an upper bound for the contact angle, and as this angle is quite low, great hydrophilicity is assured.

The very large difference in hydrophilicity between coated and uncoated PEEK can easily solve the problem presented by the visual similarity of the two. If confirmation, perhaps by a healthcare professional, of the presence of a coating is desired, a micropipette can be used. After dropping an amount of water which is small relative to the local surface of the implant, the droplet will form a tight bead on uncoated PEEK, and spread out significantly on coated PEEK. The visual difference is instantly recognizable, as seen in Figure 2-3. This provides an inexpensive and simple way to reliably determine whether a particular implant is coated, though the two appear visually identical. The patterns obtained from XRD analysis can be seen in Figure 2-4. As a
semicrystalline polymer, the PEEK substrates incorporate an XRD signature that consists of various peaks, labeled in Figure 2-4, as well as a broad region [89]. Both patterns conform well to those expected for semicrystalline PEEK. However, as can be seen from their identical shape, there is no evidence of a coating when examining XRD. This suggests that the formed coating is amorphous. This confirms the previously mentioned
Figure 2-5: SEM images at low magnification for uncoated (a) and coated (b) PEEK substrates.

ACP formation mechanism, which predicts that a thin and amorphous coating will be formed in the microwave environment. Thus, XRD analysis confirms the presence of an amorphous coating devoid of any larger crystal structure.

Surface characteristics of both uncoated and coated PEEK samples at low magnification can be seen in the SEM micrographs featured in Figure 2-5. The uncoated surface is for the most part smooth and featureless, in sharp contrast to the coated surface, where uniform coverage is seen to have occurred with no cracks present. In addition to the base coating, several spherical Ca-P globules are seen to be present. These were likely nucleated in solution and attached to the sample surface during the coating formation process. Higher magnification images can be seen in Figure 2-6, along with the EDS spectrum of the coating. The Ca/P ratio was determined to be 1.48, which is lower than the stoichiometric hydroxyapatite Ca/P value of 1.67 [90]. This is most likely due to the incorporation of substitutions in the HA structure by other ions, for example, CO$_3^{2-}$. The coating is seen to consist of many small Ca-P crystals which are needlelike in
Figure 2-6: High magnification SEM image (a) and EDS spectrum (b) of PEEK coating. Appearance. Additionally, the porosity is seen to be quite high. This small crystal size in such a thin coating supports the amorphous determination made by XRD analysis.

2.4 Conclusion

To enhance the bioactivity of PEEK for application in biomedical implants, the microwave coating process investigated offers several advantages. Contrary to all non-biomimetic approaches, the resulting coating is bonelike, containing ionic substitutions found in natural bone. This was confirmed by the relatively low Ca/P ratio seen in EDS spectroscopy. Additionally, microwave coating takes places on very short time scales, making it perfectly suitable for industrial application. The process is ideal for PEEK and many other polymers because it takes place at the relatively low temperature of 100°C. The results are promising and show great potential for the future of biomedical implants.
Chapter 3

Nitinol

3.1 Introduction

By coating the surface of an implant with a calcium phosphate (Ca-P) material, surface bioactivity can be enhanced without sacrificing advantageous bulk properties of the implant. The microwave assisted biomimetic methodology employed for polyether ether ketone (PEEK) substrates described in Chapter 2 offers many advantages over comparable approaches, including but not limited to fast coating time, bonelike hydroxyapatite (HA), low temperatures, and low cost. The favorable results obtained for titanium (Ti6Al4V) and PEEK with this approach provide justification for the investigation of the method applied to new substrate materials [76]. One such material that has recently garnered attention is nitinol, an intermetallic consisting of nickel and titanium.

Nitinol is a promising candidate for use in orthopedic implants, due to its unique shape memory properties. As a shape memory alloy, nitinol is able to recover to a previously defined shape when heated above a specific temperature [91]. This property is caused by a transition from the martensite phase present at low temperatures to the higher
temperature austenite phase [92]. In this manner, even fairly high strains of up to 6-8% can be recovered. Application of this property allows nitinol to be used for biomedical applications, such as bone clamps and sutures [93, 94]. When the devices have been heated above the activation temperature, they exert a pressure on the clamped bone as they begin to return to their previous shape. This heat activation can be achieved through several means, including warm water, ambient body temperature, or an electric pulse [91, 93, 94]. The activation temperatures vary from roughly 30-60°C, and are able to fully activate the nitinol component without undue irritation to the surrounding body tissue.

While nitinol is a unique material that may simplify and improve many medical procedures, there are concerns regarding its usage. In a similar manner to PEEK, the nitinol surface is not bioactive, and thus failure at the bone-implant interface is a possibility. In addition to this, the nickel ion Ni\(^{3+}\) is known to be toxic, and its release from the implant may cause health problems [95]. To prevent this, a coating procedure that is able to deplete the surface of Ni\(^{3+}\) ions and also support a Ca-P outer layer is necessary. This is easily obtainable by modestly adjusting the existing procedure used for PEEK substrates in Chapter 2. Immersion in \(\text{HNO}_3\) prior to NaOH etching depletes the surface of Ni\(^{3+}\) ions while also forming a TiO\(_2\) layer which will act against further corrosion [96]. This is made possible due to the passivation of Ti in \(\text{HNO}_3\) alongside the lack of passivation and subsequent release of Ni into the solution. By following this with the standard procedures already implemented for PEEK, it is possible to create a nitinol surface with both high bioactivity and low toxicity.

The elastic modulus of nitinol, which is greater than that of bone by more than a factor of ten, takes on proportionally more of the load than the surrounding bone [85, 97].
This phenomenon, known as stress shielding, may pose problems for implants constructed from solid nitinol in load-bearing applications. Underuse of bone causes a decrease in the bone density, which can potentially lead to injury. Stress shielding is also a problem for traditional titanium implants, and the situation has been remedied through the construction of porous titanium implants [98, 99]. The increased porosity compensates for the higher elastic modulus and can be used to construct an implant with the desired stiffness. In addition, the higher surface area present in porous implants provides more potential sites for bone attachment and a stronger bond at the interface. For these reasons, a porous nitinol surface is a highly promising candidate for study.

3.2 Experimental Procedure

3.2.1 Material Preparation

Nitinol substrates were produced using metal 3-D printing techniques, and were made to have a porous structure, in a grid-like pattern. The samples were cleaned in acetone, 70% ethanol, and deionized water for 10 minutes each in an ultrasonic cleaner. This was followed by air-drying overnight. All chemicals used were purchased from Fisher Scientific.

To create the functional groups needed for biomimetic deposition, as well as to limit the release of toxic Ni$^{3+}$ ions into the surrounding tissues, it is necessary to perform an alkali pre-treatment on the nitinol surface. The procedure followed by Liu et al. incorporated a pre-treatment consisting of HNO$_3$ followed by NaOH [96]. A similar procedure was followed in this study, using 32.5% HNO$_3$ to etch the surface at 60°C for 24 hours, followed by immersion in a 10 M NaOH solution at 60°C for 24 hours. For
each alkali treatment, specimens were submerged in the solution in a tightly capped bottle within a furnace set to the defined temperature. Specimens were thoroughly rinsed with deionized water between and after the treatments, and allowed to air dry overnight.

### 3.2.2 Microwave Assisted Coating

The coating procedure for the nitinol substrates is quite similar to the procedure used for PEEK. However, slight modifications were made to the coating solution in the interest of improving both coating formation and the simplicity of preparation. Due to this, the solution departs somewhat from strict biological conditions, but contains the ions necessary for the implementation of the biomimetic process. The solution was prepared by dissolving the following chemicals, respectively, into 200 mL of water: 0.6612 g Ca(NO$_3$)$_2$·4H$_2$O, 0.2016 g NaH$_2$PO$_4$, and 0.0672 g NaHCO$_3$. Chemicals were stirred for three minutes to ensure complete dissolution of each chemical before adding the next. The ionic concentration of each constituent in the coating solution can be seen in Table 3.1. This simpler composition eliminates chemicals not likely to be essential to the biomimetic formation of calcium phosphate coating. Note that the HCO$_3$− ion is still present to allow the bonelike “carbonated” apatite to form.

To begin the coating process, the samples were submerged 100 mL of the coating solution in an open glass bottle. The opening was partially covered with a piece of alumina ceramic, which trapped most of the heat inside the bottle without generating dangerously high pressures during boiling of the solution. The bottle was placed near the center of the 1200 W microwave oven (Panasonic), and irradiated for four minutes at full power. After allowing the setup one minute to cool, the samples were transferred to another glass bottle along with the remaining 100 mL of coating solution. The samples
Table 3.1: Ion concentrations of constituents of nitinol coating solution compared to those of human blood plasma [66].

<table>
<thead>
<tr>
<th>Ion</th>
<th>Human Blood Plasma</th>
<th>Nitinol Coating Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^{+}$</td>
<td>142.0</td>
<td>0</td>
</tr>
<tr>
<td>Cl$^{-}$</td>
<td>103.0</td>
<td>0</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>27.0</td>
<td>4</td>
</tr>
<tr>
<td>K$^+$</td>
<td>5.0</td>
<td>0</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>2.5</td>
<td>14.0</td>
</tr>
<tr>
<td>HPO$_4^{2-}$</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
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<td>0</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>0</td>
<td>28.0</td>
</tr>
<tr>
<td>H$_2$PO$_4^-$</td>
<td>0</td>
<td>8.4</td>
</tr>
</tbody>
</table>

were again coated in the microwave in the same manner. Because only surfaces that are openly exposed to solution are able to form the Ca-P coating, performing two repetitions ensures greater coverage. When completed, samples were gently washed with deionized water and allowed to dry in a 60°C furnace overnight.

3.2.3 Characterization

Certain methods of characterization for the nitinol samples proved problematic due to their porous structure. The presence of such large voids in the surface, while
beneficial for bone attachment, limit the amount of flat surface area for certain tests.

Water contact angle measurements cannot be performed with the equipment available, as even the smallest water droplet produced by the test machine is too large for the available surface area. Because of this, the water droplet will simply fall through the voids in the structure. Similarly, this irregular surface, coupled with the fact that the coating is quite thin, prevents the use of X-ray diffraction. However, scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) can be effectively used. To this end, the samples were examined using SEM (S4800, Hitachi), and its EDS attachment using an acceleration voltage of 10 keV. Because the base material is a conductive nickel-titanium alloy, gold coating was not necessary for the nitinol samples.

### 3.3 Results and Discussion

Unlike PEEK, the visual appearance of nitinol undergoes a noticeable change after each step of the coating procedure. Figure 3-1 illustrates the unique appearances that are present in uncoated, etched and coated forms, respectively. Uncoated nitinol is seen to have a lustrous, reflective surface, which becomes much darker and loses its reflectivity after etching. Subsequent microwave coating then alters the surface color to a lighter of the surface after coating is due to the Ca-P coating, which has a white color. In this way, a method still exists to accurately and instantly determine whether certain coating procedures have been implemented successfully, despite porous implants not allowing for the use of water contact angle measurements, as discussed above.

The surface features present for both coated and uncoated nitinol samples were investigated using SEM. At very low magnification, the porous macrostructure can be
Figure 3-1: Visual comparison of uncoated (left), MW etched (center), and MW coated (right) nitinol substrates.

seen to contain large voids before and after coating. This is shown in Figure 3-2. It is easily seen that for the coated sample, a large amount of Ca-P structure has begun to partially fill the voids. This partial filling provides a large surface area for bone attachment without compromising the benefits of the porous structure.

At higher magnification, the surface features become more distinct. Both coated and uncoated surfaces can be seen in Figure 3-3 under high magnification. The uncoated

Figure 3-2: SEM images of uncoated (a) and coated (b) porous nitinol structure at low magnification.
Figure 3-3: SEM images of uncoated (a) and coated (b) nitinol surface at high magnification.

Surface is composed of many spheres of material, formed from the 3-D printing process used to manufacture the samples. The coated image shows that the Ca-P coating shade of grey. The change in color after etching is caused by titanium oxides being formed that limit the ability of the surface to reflect light. The lighter colored appearance has uniformly covered all exposed surfaces of the sample. The number of spherical globules present, forming from amorphous Ca-P precipitation in solution and subsequent attachment to the surface, is lower than for PEEK, but nonzero. This may be due to the simpler coating solution used for the nitinol substrates. The coating itself exhibits a needle-like morphology, as is expected from previous experimentation with PEEK and titanium [76]. This structure is more easily observed in the higher magnification image shown in Figure 3-4 (a). The very small crystal size present suggests that the coating is most likely amorphous, as in the previous case of PEEK.

During SEM imaging, the EDS attachment was used to obtain an elemental spectrum for the coating. This spectrum is shown in Figure 3-4 (b). Two points are immediately obvious from this spectrum. First, the presence of calcium and phosphorous
Figure 3-4: SEM image of needle-like Ca-P coating structure (a) and associated EDS spectrum (b).

indicates that the coating is present, and the associated Ca/P ratio of 1.64 indicates that the structure is similar to hydroxyapatite (Ca/P ratio of 1.67) but slightly lower [90]. This decreased Ca/P ratio is explained by the presence of \( \text{CO}_3^{2-} \) substituting in the hydroxyapatite structure, hence the “bonelike” description applied to biomimetic coatings. Second, nickel does not appear in the spectrum. This absence is caused by both the extraction of \( \text{Ni}^{3+} \) ions during the pretreatment and subsequent covering by the Ca-P coating. In any case, the EDS data supports the determination that a coating suitable for bone attachment is present on the surface, and that there exists a low concentration of toxic \( \text{Ni}^{3+} \) ions in this coating.

3.4 Conclusion

Nitinol provides a very promising material for future use in biomedical implants. It has a number of potential applications, due to its shape memory alloy behavior. Additionally, the material may be manufactured to introduce sufficient porosity into its structure so as to tune the stiffness to be most similar to human bone. These factors provide motivation to investigate the feasibility of coating nitinol with bioactive Ca-P
materials. This study applied novel microwave assisted techniques to this end, and found that with minor changes to previous procedures, such a coating can indeed be successfully produced. While the porous morphology prevents certain characterization methods from being used, the SEM and EDS analysis confirmed that a coating similar to those previously obtained was attached to the material surface, and that toxic Ni$^{3+}$ ions were sufficiently drained from the surface, as planned. These results show that there is great promise for further study of bioactive Ca-P coatings on nitinol.
Chapter 4

Conclusion

The goal of this study was to investigate the feasibility of coating the biomedical implant materials polyether ether ketone (PEEK) and nitinol with a novel microwave-assisted biomimetic approach. This approach was first successfully applied to Ti6Al4V substrates by our research group, and the advantageous properties of PEEK and nitinol provide suitable motivation for the extension of the approach to new materials [76].

PEEK is a polymer whose elastic modulus can be altered during fabrication. By adjusting this modulus to match that of human bone, stress shielding can be eliminated [86]. This, along with its extreme inertness, makes PEEK a promising orthopedic candidate. Nitinol is a nickel-titanium intermetallic which exhibits shape memory properties. This allows it to reverse certain deformations and be used to fixate tissues as a clamp or suture [92-94]. Though its elastic modulus is much higher than bone, introducing a porous structure can adjust the stiffness to approximate that of bone, and reduce stress shielding.

Neither PEEK nor nitinol are bioactive without a surface treatment, and thus require a surface modification prior to application. A calcium phosphate (Ca-P) coating enhances bioactivity, allowing bone to bond directly to the implant surface. In the case of nitinol, this process can remove toxic Ni$^{3+}$ ions near the surface before they come into
contact with the body. The biomimetic approach can provide high quality thick coatings that mimic actual bone chemistry, but are unfortunately quite time consuming, on the order of days or weeks. By introducing microwave irradiation to the biomimetic methodology, coating time is drastically reduced to minutes. This allows coating of PEEK and nitinol in a manner efficient enough to be applied in industry.

Coatings obtained in this way were examined using scanning electron microscopy (SEM) and were seen to provide complete coverage of the implant, even in the case of porous nitinol. The calcium to phosphate ratio was found using energy dispersive spectroscopy (EDS) to be consistent with values expected for bone-like Ca-P, which contains substitutions of other ions, such as $\text{CO}_3^{2-}$. This coating visually appeared to be amorphous, and in the case of PEEK, this was confirmed through X-ray diffraction (XRD). To ensure the presence of a coating, visual examination of the water contact angle and surface coloring proved sufficient for PEEK and nitinol, respectively.

The coatings obtained using this microwave-assisted biomimetic technique were found to be of suitable quality to warrant further investigation. Because the process is simple and fast, this microwave-assisted process is well-suited to industrial applications. For this to happen, the behavior of the coatings in animal and eventually human studies must be investigated. Future work should focus on these trials and in optimizing the coatings for biomedical application.
References


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