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Mechanical Characterization of Chitosan Coating Deposited Using Electrospray Method

Ewe Jiun Chng

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MECHANICAL CHARACTERIZATION OF CHITOSAN COATING DEPOSITED USING ELECTROSPRAY METHOD

By

Ewe Jiun Chng

A Thesis

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science

Major: Mechanical Engineering

The University of Memphis

May 2018
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ABSTRACT

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Characterization of Chitosan Coatings Deposited Using Electrospray and Solution Casting Methods. Major Professor: Ranganathan Gopalakrishnan, Ph.D.

Incorporation of coating to enhance the bioactivity, anti-microbial and drug delivery capability of implant has been the interest of researchers. Chitosan is a biopolymer that exhibits these properties. In this study, electrospray method was used to coat 2D titanium substrate and titanium screw with chitosan. The focus was to compare the bond strength between the coatings produced using electrospray and solution casting method. The effect of silane-based treatment to chemically bond chitosan to the surface of titanium for better bonding was evaluated. The bond strength of the 2D coatings was evaluated using tensile and shear tests. The coating retention on screw was evaluated using functional bone simulation test. Tensile test shows higher tensile bond strength from electrospray coating compared to solution cast coating. No significant strength difference was observed between silane-based treated and non-treated coatings. Functional bone simulation test shows average of 12% loss in coating mass on the screw.
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<thead>
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<tr>
<td>%</td>
<td>Percent</td>
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<tr>
<td>°</td>
<td>Degree</td>
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<tr>
<td>°C</td>
<td>Degree Celsius</td>
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<tr>
<td>µl</td>
<td>Microliter</td>
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<td>µm</td>
<td>Micrometer</td>
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<tr>
<td>µS</td>
<td>Millisiemen</td>
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<td>2D</td>
<td>Two dimensional</td>
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<tr>
<td>3D</td>
<td>Three dimensional</td>
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<td>CI</td>
<td>Confidence interval</td>
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<td>cm</td>
<td>Centimeter</td>
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<td>et al.</td>
<td>and others</td>
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<tr>
<td>FTIR</td>
<td>Fourier-transform infrared spectroscopy</td>
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<td>g</td>
<td>Gram</td>
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<td>hrs</td>
<td>Hours</td>
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<tr>
<td>ID</td>
<td>Internal diameter</td>
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<tr>
<td>kDa</td>
<td>Kilodalton</td>
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<td>kg/m³</td>
<td>Kilogram per cubic meter</td>
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<td>kPa</td>
<td>Kilopascal</td>
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<td>kV</td>
<td>Kilovolt</td>
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<td>Symbol</td>
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<td>M</td>
<td>Mole</td>
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<td>mbar</td>
<td>Millibar</td>
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<td>mm</td>
<td>Millimeter</td>
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<tr>
<td>MPa</td>
<td>Megapascal</td>
</tr>
<tr>
<td>Non</td>
<td>Not treated by silane</td>
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<tr>
<td>-OH</td>
<td>Hydroxyl</td>
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<tr>
<td>PLA</td>
<td>Poly(lactic) acid</td>
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<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate)</td>
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<tr>
<td>rpm</td>
<td>Round per minute</td>
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<tr>
<td>s</td>
<td>Second</td>
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<tr>
<td>SiC</td>
<td>Silicon carbide</td>
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<td>Silanated</td>
<td>Silane-based treated</td>
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<td>Silanation</td>
<td>Silane-based treatment</td>
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<td>Silane</td>
<td>Tri-ethoxy-silylbutyraldehyde (TESBA) silane</td>
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<tr>
<td>Std dev</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume by volume</td>
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vol% Percentage by volume
1. Introduction

1.1. Chitosan & Its Applications

Biomedical implant devices for dental/craniofacial and orthopedic applications are a reliable and effective means for repairing/re-storing function of damaged, diseased or missing tissues. Despite success of these devices, there are still challenges in the use of these devices with respect to improving their integration into boney tissues, promoting healing and resisting/preventing infection. Numerous research efforts have been dedicated in looking for suitable implant coating material that would improve the biocompatibility, osseointegration, bacteriostatic and drug delivery capability of implant. Calcium phosphate, bioactive glass, poly(methyl methacrylate) (PMMA) and poly(lactic) acid (PLA) are materials used as implant coatings [1-6]. Although calcium phosphate and bioactive glass exhibit excellent biocompatibility property, their brittle nature will lead to interfacial fracture between coating and substrate when subjected to shear loading [1, 7]. Furthermore, calcium phosphate and PMMA do not exhibit bacteriostatic property which could lead to bacterial infection at implant insertion site due to the adhesion of bacteria on the implant coating [2, 8]. PLA is widely used in medical field due to its biocompatibility, biodegradable and non-toxic nature of degradation products [6]. However, such synthetic polymers exhibit hydrophobic nature which will inhibit cell adhesion and growth [9].

Chitosan is a linear polysaccharide derived from the deacetylation of chitin polymer which can be found in arthropod exoskeletons [10]. Chitosan has piqued the interest of researchers all over the world due to its biocompatibility, biodegradability, bioadhesivity, bacteriostatic and drug delivery capability.
Chitosan biocompatibility has been reported in numerous studies showing minimal foreign body reaction to the chitosan-based implants, improvement in wound healing and cell proliferation [12-15]. For example, a study of wound healing on rat using chitosan film by Denuziere et al. has demonstrated that wound treated with chitosan film cured at a faster pace compared to wound not treated with chitosan film [12]. Besides, throughout the treatment period, no abnormal inflammatory reaction or toxicity was observed from the animals [12]. The biodegradation of chitosan is through enzymatic hydrolysis mainly by the enzyme called lysozyme [16]. The breakdown products (amino sugars) of chitosan is non-hazardous and can be absorbed/metabolized by human body [17]. Next, chitosan has also shown its bacteriostatic ability to curb the growth of various microorganisms such as fungi and bacteria [2, 18, 19]. Greene et al. reported a zone of inhibition from S. aureus was detected on stainless steel screw coated with chitosan even though the chitosan coating was not loaded with gentamicin (antibiotic) [2]. This shows the ability of chitosan coating inhibiting the growth of bacteria in

**Fig. 1. Polymers of chitin and chitosan [11]**
close vicinity. The exact antibacterial mechanisms of chitosan is still unknown, it is thought that the positively charged chitosan molecules and negatively charged bacterial cell membranes changes the bacteria cell permeability [20]. This would inhibit the transportation of essential solutes into the bacteria cell and results in leakage of proteinaceous, thus killing the bacteria [20]. Bacterial infection is one of the complication arises after implant surgery due to bacteria adhesion on implant surface causing biofilm formation on implantation site. This may lead to implant replacement, amputation and mortality [21]. This shows the need of antimicrobial coating such as chitosan on implant to reduce risk of biofilm formation after implant insertion.

Another interesting property of chitosan is its ability to encapsulate and release drugs, proteins and gene products [2, 17, 22-28]. This ability originates from the positively charged nature of chitosan molecule and its high charge density in solution [10]. The charge density of the chitosan molecule enables the binding with water-soluble anionic polymers and drugs forming insoluble ionic complexes [10, 29]. The charge density of the chitosan is dependent on pH, under physiological pH will result in dissociation of a portion of the immobilized polyanion [10]. This process enables chitosan to act as bioactive materials carrier for localized delivery. For example, Arya et al. has demonstrated the encapsulation and release of ampicillin using chitosan micro/nanospheres over duration of 5 days [17]. Qin et al. deposited chitosan loaded with bone morphogenetic proteins (rhBMP-2) to improve the surface bioactivity and osseointegration of titanium [23]. Roy et al. has demonstrated the use of chitosan to deliver plasmid DNA for oral immunization [27]. These properties have made chitosan a good candidate for implant coating which could facilitates cell adhesion, wound healing and drug delivery.
1.2. *Electrospray Deposition Method*

Numerous techniques have been developed to coat metal and ceramics with chitosan. These techniques can be categorized into passive and electrostatic methods as summarized by Avilez *et al.* [30]. Passive methods are freeze drying, solution casting and spin coating while electrostatic methods are layer-by-layer, electrolytic and electrophoretic deposition. Freeze drying method involves dipping the substrate in chitosan solution then freeze it before placing the coated substrate in vacuum chamber at low temperature to induce sublimation of the solvent used in chitosan solution. Solution casting involves casting chitosan solution on the substrate surface and allowing the solvent to dry under room temperature or in a heated environment. The thickness of the coating can be controlled by using carrier tape or doctor blade to spread or cast the chitosan solution in certain thickness. Spin coating rotates the substrate deposited with chitosan solution at a constant angular velocity. The centripetal force will spread the chitosan uniformly on the substrate and excessive solution will be thrown off as shown in Fig. 2. The disadvantages of these passive methods are difficult to uniformly coat 3D object with irregular contour, difficult to control the thickness of the coating and the excessive chitosan solution used is not recoverable. Although, carrier tape or doctor tape can be used to control the thickness and uniformity of coating, this method is effective on 2D plain surface but not 3D surfaces with complex contour.
Fig. 2. Stages of the spin coating process [31]

Electrostatic methods provide a better way at coating 3D object with complex contour by utilizing electric charge. Layer-by-layer technique coats the substrate by joining alternating layers of polycation and polyanion using electrostatic interaction as shown in Fig. 3.

Fig. 3. Layer-by-layer coating process [32]

Electrolytic deposition passes electrical current through chitosan solution between cathode and anode where the substrate acts as cathode. Due to the positive charge nature of
chitosan molecule, it will be attracted towards the cathode/substrate and precipitate on the cathode/substrate surface forming a layer of coating. Wang et al. demonstrated the deposition of chitosan/gelatin/nanosilver on different types of cathode/substrate using electrolytic deposition as shown in Fig. 4.

![Fig. 4. Electrolytic deposition of chitosan/gelatin/nanosilver on cathode/substrate made of different metals [33]](image)

On the other hand, electrophoretic deposition technique deposits electrically charged particles suspended in liquid medium onto a substrate using external electric field. Although electrostatic methods are better at coating 3D surfaces with complex contour compared to passive methods, they still pose several disadvantages. Firstly, it is difficult to control the thickness of the coating due to the decrease in chitosan concentration over time as more chitosan gets precipitated, it will require precise and accurate timing to achieve the desired thickness of coating. Secondly, the coating process requires precise voltage control to achieve optimum coating morphology or high voltage will induce surface crack. Next, post processing on the coating may be required to get thin and uniform surface. Furthermore, the process will generate excessive wastage since some of the chitosan solution is not recoverable.

One approach to overcome these drawbacks as mentioned is electrospray deposition technique. Electrospray deposition is also known as electrohydrodynamic atomization.
Electrospray utilizes the principle of particle/droplet charging to produce coating. Fig. 5 shows the setup to coat the aluminium substrate with chitosan using electrospray method.

**Fig. 5.** Schematic diagram of electrospray deposition of chitosan on aluminium substrate [34]

Chitosan solution is pushed out from the nozzle by the applied pressure from the syringe pump. The nozzle is connected to positive high voltage source to charge the chitosan solution as well as creating a high electric field between the nozzle and substrate. As the chitosan droplet emerge from the nozzle, the electric field applied will deform the interface of the droplet [35]. The electric charge generates electrostatic repulsion force within the droplet. The maximum number of electric charge a droplet or particle could carry is called Rayleigh limit. If the number of charge in a droplet exceeds its Rayleigh limit, electrostatic repulsion force overcomes the surface tension of the droplet, the excess charge will be dissipated through the breakup of large droplet into micro to nano-sized droplets. Since the micro/nano sized droplets carry similar charge, the Coulomb repulsion between the droplets will disperse and not reaccumulate together. The electric field between the nozzle and substrate drives the micro/nano sized droplets towards the substrate. The advantages of electrospray are: easy to coat 3D surfaces with complex and irregular contour by utilizing electric charge, precise control on coating thickness, capable of controlling the size distribution of the sprayed droplets and produces less wastage. One major
drawback of electrospray method is the low throughput of single capillary electrospray. This drawback can be overcome by using multi-nozzles electrospray for higher throughput [36].

Several studies have been conducted to investigate the application of electrospray deposition method on chitosan-based coating to improve the osseointegration on implant and the generation of micro/nano sized chitosan particles encapsulated with bioactive materials such as drugs for drug delivery application [17, 23-26, 37]. Most of the studies were focused on evaluating the biological response and performance of the chitosan coatings, such as cell culture test, drug release profile evaluation, in vivo test and in vitro test than focused on the coating strength. Besides, these tests were done on coatings of 2D substrate and not on 3D substrate. Based on the literature review, electrospray is not a novel technique used to produce chitosan-based coating, however, to realize the use of chitosan coating for clinical applications, the bond strength and mechanical properties between the coating and substrate need to be studied thoroughly.

1.3. Silane-based Treatment (Silanation) on Substrate Surface for Better Bonding

The aim of this silane-based treatment is to chemically bond the coated chitosan to the substrate for better bond strength. This method has been evaluated by Bumgardner et al. and the study shows three times increase in bond strength on the silane-based treated (silanated) (1.5 – 1.8 MPa) and non-treated coatings (non-silanated) (0.5 MPa) [1]. The silane-based treatment (silanation) involves depositing tri-ethoxy-silylbutyraldehyde (TESBA) silane on the surface of titanium substrate to form a reactive aldehyde group. Chitosan deposited on the titanium surface is covalently bonded through imine bond between the aldehyde group on the titanium surface and amino group of the chitosan polymer. Fig. 6 shows the reaction between silane, surface of titanium substrate and chitosan.
Fig. 6. Step (1): Silanation reaction between titanium surface and tri-ethoxy-silylbutyraldehyde (TESBA). Step (2): Reaction of silane with chitosan [38]

1.4. Motivation and Research Goal

The motivation for this research is the advantage of chitosan as the coating material and electrospray method over other materials and techniques. To achieve the goal as mentioned, there are certain factors to be investigated. This leads to following research goals, which we will try to address in this work:

i. To evaluate the effectiveness of electrospray method as a delivery technique for chitosan coating on 2D and 3D surfaces of implant.

ii. To evaluate and compare the bond strength of chitosan coating deposited by electrospray and solution casting methods.

iii. To evaluate and compare the coating strength between silanated and non-silanated coatings
2. Electrospay Deposition of Chitosan on 2D Surface

2.1. Optimization of Spray Parameters

The spray parameters were optimized to achieve balanced and desirable outcome between spray stability, spray flowrate and spray conditions. The spray stability refers to the ability of the electrospay sustaining the stability of the spray without interruption such as large droplet formation or accumulation of spray solution on the nozzle. The spray flowrate refers to the rate of volume of the chitosan solution can be ejected from the electrospay and spray conditions refer to dry or wet deposition of the chitosan droplets on the substrate. The spray parameters with their effect on the outcomes investigated are as listed below.

2.2. Electrospay Parameters

2.2.1. Voltage

The voltage difference between the nozzle of the capillary and the grounded substrate determines the strength of electric field between these 2 points and the number of charge per droplet holds. Increasing the voltage enables higher spray flowrate (volume per min) of the chitosan solution thus shorten the coating time. However, precaution must be taken to ensure that the voltage between the nozzle of the capillary and grounded substrate does not exceed the breakdown voltage of the air to prevent electrical arc formation which could cause localized heating and potentially change the chemical properties of the chitosan.

2.2.2. Nozzle to Substrate Distance

The distance between the nozzle to substrate determines the flight time of the droplets from the nozzle to the substrate. Larger distance implies that droplets will need to travel for a longer time in the air causing them to dry up more thus affects the spray condition of the coating. Preliminary strength test of the chitosan coatings produced by dry deposition shows high
inconsistency and lower bond strength compared to the coatings produced by wet deposition. As the chitosan coatings are produced layer by layer using electrospray method, dry deposition of dry chitosan droplet/particle may cause improper adhesion/bonding between each subsequent layer. Furthermore, the formation of gaps and cracks in the coating due to dry deposition may cause the inconsistency of coating bond strength. The nozzle to substrate distance was adjusted to ensure the chitosan droplet/particle remains wet upon deposited on the substrate while the gap is enough to ensure no voltage breakdown.

2.2.3. Capillary

The internal diameter of the capillary is one of the main factor in determining the spray flowrate. Larger internal diameter of the capillary will enable higher spray flowrate because the pressure drop across the capillary is low. Furthermore, the length of the capillary is one of the factor affecting the spray flowrate because long capillary will induce more pressure drop hence lower spray flowrate. Silica and nickel type capillary were tested to evaluate the spray stability. Nickel capillary offers better spray stability when compared to silica capillary due to its conductivity while silica is non-conductive. As a result, silica capillary forms a higher electric field strength between its nozzle and substrate therefore, the better spray stability. However, nickel capillary is not suitable for biomedical application as nickel itself is harmful to health. Silica capillary on the other hand doesn’t not poses any harmful effect to health. The nozzle of the silica capillary needs to be cleaned and dried before each spray to ensure good spray stability. Deposition of chitosan on the nozzle over time during the spray process will increase the hydrophilicity on the nozzle causing chitosan solution to accumulate on it which will compromise the spray stability leading to large droplet formation during the spray process.
2.2.4. Pressure

Pressure supplied to the electrospray determines the spray flowrate. Higher pressure will push the chitosan solution through the capillary at a higher rate and vice versa. The level of pressure supplied is dependent on the pressure drop across the capillary.

2.3. Chitosan Solution Parameters

2.3.1. Chitosan Percentage

The chitosan percentage affects the viscosity of the solution. The higher the percentage content, the higher the viscosity of the solution. Higher viscosity of the solution will increase the pressure drop across the capillary therefore, reduce the spray flowrate.

2.3.2. Reagent Alcohol Percentage

The addition of reagent alcohol increases the spray stability of the chitosan solution by reducing its viscosity and surface tension. However, high alcohol content in the chitosan solution will increase the chance of clogging the nozzle of the capillary due to the solution drying up at a higher rate because of the alcohol high volatility.

The optimum spray parameters were determined using trial and error method.
2.4. Methodology

2.4.1. Materials

Titanium substrates (Titanium Industries, commercially pure ASTM F67) with dimension of $25 \times 25 \times 1 \text{ mm}$ were used for tensile test. Titanium substrates (Titanium Industries, commercially pure ASTM F67) with dimension of $19 \times 65 \times 1 \text{ mm}$ were used for shear test. 92.6% de-acetylated chitosan powder (Heppe Medical Chitosan GmbH, product no.: 24711) with molecular weight of 300 – 700 kDa was used to prepare the chitosan solution. Reagent alcohol (VWR Analytics, product no.: BDH1156-4LP) containing 95% ethanol + methanol and 5% 2-propanol solution was used to dilute the chitosan solution and improve its sprayability.

2.4.2. Cleaning and Passivation of Substrate

Titanium substrates for tensile and shear test were wet grounded with sequence of 400, 600, 800 and 1200 grit SiC paper using a grinder/polisher (Buehler, Metaserv 2000). The grounded substrates were cleaned with alconox detergent and warm water. Next, the substrates were ultrasonically cleaned with acetone, ethanol and de-ionized water for 10 min each and passivated using 70:30 vol% de-ionized water/nitric acid for 30 mins at ambient conditions. The passivated substrates were then rinsed with de-ionized water and placed in a covered pure water bath for 24 hrs for the formation of –OH groups on the surface of the substrates.

2.4.3. Silanation of Substrate

The silanation process of the titanium substrate was adopted from Bumgardner et al. [1]. First, the titanium substrates were suspended in a stirred 5:95 vol% de-ionized water/ethanol solution maintained at pH 4.5 with 10M acetic acid and 1M sodium hydroxide. Tri-ethoxy-silylbutyraldehyde (TESBA) silane was added to make a 2% (v/v) solution of silane in ethanol solution. The titanium substrates were left on a belly dancer for 10 mins to react with silane.
which the ethoxy groups of the silane molecules are organized via H-bonding with oxides and hydroxide group on the titanium substrate surface. Non-silanated substrates were omitted the addition of silane. The silanated substrates were rinsed with ethanol to remove non-adhered silane and cured at 110 °C for 10 mins to convert the H-bond between silane and titanium to covalent Si-O bonds.

2.4.4. Storage of Silanated Substrate

The silanated substrates were immediately stored in a vacuum chamber after being cured in the oven. First, the air in the chamber was drawn out using a vacuum pump (Ted Pella, Value VRI-2 Rotary) to pressure range of 10 – 2 mbar. Next, nitrogen gas was flushed into the evacuated chamber and the gas in vacuum chamber was again drawn out. This is to minimize the oxygen content in the vacuum chamber to prevent the silane on titanium substrate being compromised by reacting to oxygen. These steps were repeated every time the vacuum chamber was opened.

2.4.5. Chitosan Solution Preparation

1% chitosan solution with 0.5% acetic acid was used to electrospray the chitosan coating on the titanium substrate. The chitosan solution was made by dissolving 92.6% de-acetylated chitosan powder with molecular weight of 300 – 700 kDa in 0.5% acetic acid at room temperature stirred using a magnetic stirrer. Prior to the spray, reagent alcohol with 95% ethanol + methanol and 5% 2-propanol solution was added to reduce the viscosity as well as surface tension of the chitosan solution to make it sprayable. The final solution contained 25:75 vol% reagent alcohol/chitosan solution. The solution was stirred using magnetic stirrer to ensure reagent alcohol and chitosan mixed thoroughly. The pH value of the final solution is measured to
be 5.1 and conductivity of 908 µS. The mixed solution is sonicated to remove microbubble in the solution before being sprayed.

2.4.6. Electrospray Process

The electrospray setup used was as shown in Fig. 7 and Fig. 8. A single capillary electrospray as shown in Fig. 9 was used to coat the substrates. The electrospray contains 3 inlets and 1 outlet which are pressure inlet, material inlet, high voltage inlet and capillary outlet. Pressurized air was fed into the electrospray from air compressor and pressure regulator unit to push the chitosan solution out from the electrospray through a capillary. The chitosan solution was stored in a vial attached to the electrospray. The positive output of high voltage regulator (EMCO, E80CTAB) was connected to the electrospray through high voltage BNC cable to charge the chitosan solution to a high voltage through the metal conductor submerged in the chitosan solution in the vial shown in Fig. 9 while the negative terminal (ground) was connected to substrate as shown in Fig. 7. Capillary submerged in the chitosan solution in the vial delivers the solution out of the electrospray. The spray parameters used are summarized in Table 1 below.

Table 1

Spray parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Output voltage of high voltage regulator</td>
<td>6.3 kV</td>
</tr>
<tr>
<td>Capillary tip to substrate distance</td>
<td>3 mm</td>
</tr>
<tr>
<td>Capillary type, length and ID</td>
<td>Silica, 38 cm, 250 µm</td>
</tr>
<tr>
<td>Spray flowrate</td>
<td>38 µl/min</td>
</tr>
<tr>
<td>Spray time</td>
<td>33 mins</td>
</tr>
<tr>
<td>Spray volume per substrate</td>
<td>1.25 ml</td>
</tr>
</tbody>
</table>

The tip of the silica capillary was grinded to ensure it is smooth and flat to improve the stability of the spray. Prior to remove the substrate in the vacuum chamber, nitrogen gas was flushed into the vacuum chamber to minimize the exposure of the substrates to oxygen in the air.
Air was then drawn out after substrate was taken out to preserve the other substrates in the vacuum chamber. The substrate was mounted on the translation stage using tweezer to prevent contact on the silanated surface causing contamination. Once the substrate is mounted and grounded to the negative terminal of the high voltage regulator, chitosan solution was sprayed immediately to coat the silanated surface and prevent oxygen in the air from compromising the silane on the surface. The electrospray process was carried out under ambient conditions. The charged chitosan solution emerging from the capillary tip breaks up into charged droplets and the electric field between the capillary tip and substrate drives the chitosan droplets towards the substrate as shown in Fig. 10. The linear translation stage moves the substrate in x and y direction to coat the surface area of the substrates used for tensile and shear test. Surface area of 625 mm\(^2\) was coated on the substrates used for tensile (fully coated, 25 × 25 mm) and shear test (partially coated, 19 × 32.9 mm). The 2D chitosan coating was sprayed with the pattern as shown in Fig. 11. The spray pattern is divided into 8 stages with each stage followed by the subsequence stage (1 → 2 → 3 → … → 8 → 1 → 2 …). The number of division in x and y axis was divided such that the gap distance between each parallel spray path has less than or equal to 2.30 mm. The speed of each axis of the translation stage was set at 12.2 mm/s. The coating time was approximately 33 mins, delivering 1.25 ml of chitosan solution per coating. The coated substrates with liquid chitosan on them were carefully removed from the translation stage and stored in a petri dish for 4 days to dry before neutralized and tested.
Fig. 7. Schematic of electrospay setup

Fig. 8. Actual electrospay setup
Fig. 9. Diagram of electrospray unit

Fig. 10. Interaction of chitosan droplets between capillary tip and substrate
2.4.7. Solution Cast Process

The solution casting process was carried out at ambient conditions. 1.25 ml of chitosan solution was extracted using a pipette and casted over the titanium substrates. Precaution was taken to ensure the chitosan solution did not overflow from the titanium substrate and the entire surface is casted to ensure uniform thickness of the coating. The substrates were left for 4 days to dry before neutralized and tested.

2.4.8. Neutralization

The coated substrates were neutralized to remove the acetic acid in the coating prior to test. The substrates were suspended in 0.25 M phosphate buffer solution and placed on belly dancer for 20 mins. The substrates were then rinsed with de-ionized water and left for 24 hours to dry before being tested.
2.4.9. *Tensile Bond Strength Test*

Tensile test was carried out to determine the tensile strength of the silanated and non-silanated chitosan coatings produced by electrospray and solution casting method using mechanical testing machine (Instron, model 4465). Aluminium stud with diameter of 11.5 mm was glued at the center of the coatings using epoxy (3M, Scotch-Weld DP405). The surface of the aluminium studs were grinded with 120 grit SiC paper to increase the surface area for the epoxy to bond to, thus increasing the bond strength between the aluminium stud and epoxy interface. The epoxy was cured in an incubator at curing temperature of 37 °C and curing pressure of 12 kPa for 24 hrs before tested. Fig. 12 shows the tensile test setup of the chitosan coated titanium substrate mounted on the mechanical testing machine. A custom-made fixture was used to hold the substrate in place. The aluminium stud glued to the chitosan coating was pinned to the crosshead of the machine. The tensile test on chitosan coatings were conducted with displacement rate of 0.5 mm/min and force/displacement data acquisition data rate of 20 points/s. The maximum load recorded was converted to stress in MPa using the cross-sectional area of the aluminium stud. The tested coatings were visually inspected to determine at which interfaces (epoxy-stud, epoxy-coating, coating-substrate) the fracture occurred.
2.4.10. Shear Bond Strength Test

Shear test was carried out to determine the shear strength of the silanated and non-silanated chitosan coatings produced by electrospray and solution casting method using mechanical testing machine (Instron, model 4465). Aluminium coupons with dimension of 18.8 × 75 × 1 mm were prepared and glued to the coatings on the titanium substrate using epoxy (3M, Scotch-Weld DP405). The glued area was 18.8 × 30 mm giving an area of 564 mm². The surface of the aluminium coupons were grinded with 120 grit SiC paper to increase the surface area for the epoxy to bond to, thus increasing the bond strength between the aluminium coupons and epoxy interface. The epoxy was cured in an incubator at curing temperature of 37 °C and curing pressure of 35 kPa for 24 hrs before tested. Fig. 13 shows the shear test setup of the chitosan coated titanium substrate glued to the aluminium coupon mounted on the mechanical testing machine. Two pneumatic grips, clamped on both ends of the aluminium coupon and titanium substrate were to hold the glued titanium substrate and aluminium coupon in place and pull them apart. The shear test on chitosan coatings were
conducted with displacement rate of 0.5 mm/min and force/displacement data acquisition data rate of 20 points/s. The maximum load recorded was converted to stress in MPa using the glued area between the coating on titanium substrate and aluminium coupon which is $564 \, mm^2$. The tested coatings were visually inspected to determine at which interfaces (epoxy-coupon, epoxy-coating, coating-substrate) the fracture occur.

**Fig. 13.** Shear test setup of chitosan coated titanium substrate and aluminium coupon on pneumatic grips and Instron mechanical testing machine.
2.5. Results

2.5.1. Coating on Titanium Substrate

Coatings produced using electrospray and solution casting methods exhibit similar visual appearance. As shown in Fig. 14, the chitosan coating on the titanium substrate shows a transparent and smooth outlook. The thickness of the coatings was measured to be approximately 16 µm using micrometer.

![Comparison between coated and non-coated area](image)

**Fig. 14.** Comparison between coated and non-coated area

Scanning electron microscopy was done to examine the surface of the chitosan coatings produced from electrospray and solution casting as shown in Fig. 15 below. Both images show a smooth and uniform chitosan coating on the titanium substrate.
Fig. 15. SEM image of solution cast and electrospray coatings
2.5.2. *Tensile Bond Strength Test*

Tensile test was done on silanated and non-silanated chitosan coating produced using electrospray and solution casting methods. The number of samples tested for each case is listed in Table 2 below.

**Table 2**

Number of samples for each tensile test case

<table>
<thead>
<tr>
<th>Cases</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrospray, non-silanated</td>
<td>16</td>
</tr>
<tr>
<td>Electrospray, silanated</td>
<td>16</td>
</tr>
<tr>
<td>Solution cast, non-silanated</td>
<td>6</td>
</tr>
<tr>
<td>Solution cast, silanated</td>
<td>6</td>
</tr>
</tbody>
</table>

It was observed that the tensile bond strength of the chitosan coating is affected by the displacement rate of the tensile test. Higher displacement rate will result in lower tensile bond strength. Demo tensile test was carried out to determine the appropriate displacement rate for the test. It was observed that displacement rate of 0.5 mm/min or lower didn’t contribute to significant variability in the tensile bond strength, therefore, displacement rate of 0.5 mm/min was used to conduct the tensile test.

Each of the test sample subjected to the tensile test was examine visually to confirm the chitosan coating fail at the coating-substrate interface. An alternative way of checking is by peeling the coating off from the side of the substrate by using a strong adhesive tape. The peeled off coating from the side can be slowly peeled towards the centre to check if there is any coating still intact. Based on the outcome of the tensile test, the 3M Scotch-Weld DP405 epoxy together with 120 grit grinded aluminium stud has sufficient bond strength to peel the coating off the
substrate as all the coatings tested failed at the coating-substrate interface. Fig. 16 shows the peeled off area after the tensile test.

**Fig. 16.** Peeled chitosan coating after tensile test

The tensile bond strength of the tested chitosan coatings is shown in Table 3 and Fig. 17.

**Table 3**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Electrospray (MPa)</th>
<th>Solution Cast (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Silanated</td>
<td>Non-silanated</td>
</tr>
<tr>
<td>1</td>
<td>3.03</td>
<td>2.63</td>
</tr>
<tr>
<td>2</td>
<td>2.39</td>
<td>2.00</td>
</tr>
<tr>
<td>3</td>
<td>3.23</td>
<td>1.60</td>
</tr>
<tr>
<td>4</td>
<td>3.70</td>
<td>1.17</td>
</tr>
<tr>
<td>5</td>
<td>2.64</td>
<td>1.74</td>
</tr>
<tr>
<td>6</td>
<td>1.45</td>
<td>2.72</td>
</tr>
<tr>
<td>7</td>
<td>1.52</td>
<td>3.81</td>
</tr>
<tr>
<td>8</td>
<td>1.49</td>
<td>2.14</td>
</tr>
<tr>
<td>9</td>
<td>1.94</td>
<td>2.74</td>
</tr>
<tr>
<td>10</td>
<td>2.15</td>
<td>2.42</td>
</tr>
<tr>
<td>11</td>
<td>0.58</td>
<td>4.76</td>
</tr>
<tr>
<td>12</td>
<td>2.16</td>
<td>1.54</td>
</tr>
<tr>
<td>13</td>
<td>1.71</td>
<td>3.75</td>
</tr>
<tr>
<td>14</td>
<td>3.15</td>
<td>2.38</td>
</tr>
<tr>
<td>15</td>
<td>2.14</td>
<td>1.70</td>
</tr>
<tr>
<td>16</td>
<td>2.54</td>
<td>3.04</td>
</tr>
<tr>
<td>Mean</td>
<td>2.24</td>
<td>2.51</td>
</tr>
<tr>
<td>Std Dev</td>
<td>0.80</td>
<td>0.96</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.81 – 2.67</td>
<td>2.00 – 3.02</td>
</tr>
</tbody>
</table>
Fig. 17. Bar graph of the tensile bond strength (MPa) of different coatings with 95% confidence interval

The error bars in Fig. 17 represent the 95% confidence interval calculated based on student’s t-distribution. Analysis of variance and Tukey’s post hoc test (significant level, $\alpha = 0.05$) indicated no significant tensile bond strength difference between silanated and non-silanated coatings produced using electrospray and solution casting method. However, there were significant differences between the tensile bond strength of electrospray and solution casting coatings for both silanated and non-silanated. The coatings produced by electrospray method have higher tensile bond strength compared to solution casting method.

2.5.3. Shear Bond Strength Test

Shear test was done on silanated and non-silanated chitosan coatings produced using electrospray and non-silanated coatings by solution casting method. Silanated coating produced by solution cast method was not tested because the effect of silane was not significant according
to the tensile bond strength test result as well as the shear bond strength test result of silanated and non-silanated coatings produced by electrospray method as shown in Fig. 19. The number of samples tested for each case is listed in Table 4 below.

**Table 4**

Number of samples for each shear test case

<table>
<thead>
<tr>
<th>Cases</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrospray, non-silanated</td>
<td>5</td>
</tr>
<tr>
<td>Electrospray, silanated</td>
<td>5</td>
</tr>
<tr>
<td>Solution cast, non-silanated</td>
<td>5</td>
</tr>
</tbody>
</table>

Each of the test sample subjected to the shear test was examine visually or physically using adhesive tape as mentioned to confirm the chitosan coating fail at the coating-substrate interface. Based on the outcome of the shear test, the 3M Scotch-Weld DP405 epoxy together with 120 grit grinded aluminium coupon has sufficient bond strength to peel the coating off the substrate as all the coatings tested failed at the coating-substrate interface. Fig. 18 shows the peeled off area after the shear test.

![Fig. 18. Remaining coating and peeled coating after shear test](image-url)
The peeled and unpeeled area of the coating can be identified visually through the difference in color. The shear bond strength of the tested chitosan coatings is shown in Table 5 and Fig. 19.

**Table 5**

Shear bond strength of electrospray and solution cast coating

<table>
<thead>
<tr>
<th>Sample</th>
<th>Electrospray (MPa)</th>
<th>Solution Cast (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Silanated</td>
<td>Non-silanated</td>
</tr>
<tr>
<td>1</td>
<td>4.83</td>
<td>3.50</td>
</tr>
<tr>
<td>2</td>
<td>4.64</td>
<td>5.33</td>
</tr>
<tr>
<td>3</td>
<td>4.49</td>
<td>5.15</td>
</tr>
<tr>
<td>4</td>
<td>3.70</td>
<td>3.37</td>
</tr>
<tr>
<td>5</td>
<td>5.73</td>
<td>3.72</td>
</tr>
<tr>
<td>Mean</td>
<td>4.68</td>
<td>4.21</td>
</tr>
<tr>
<td>Std Dev</td>
<td>0.73</td>
<td>0.95</td>
</tr>
<tr>
<td>95% CI</td>
<td>3.78 – 5.58</td>
<td>3.03 – 5.39</td>
</tr>
</tbody>
</table>

**Comparison of Shear Bond Strength (MPa) Between Different Coatings**

**Fig. 19.** Bar graph of the shear bond strength (MPa) of different coatings with 95% confidence interval
The error bars in Fig. 19 represent the 95% confidence interval calculated based on student’s t-distribution. Analysis of variance (significant level, $\alpha = 0.05$) indicated no significant shear bond strength difference between these 3 groups.

2.6. Discussion

The aim of this research was to investigate and compare the mechanical strength of the chitosan coating produced using electrospray and solution cast method based on tensile and shear strength tests. To determine the absolute strength of the coating, the adhesive used must have higher coating-adhesive bond strength than coating-substrate bond strength. Several adhesives were tested and the 3M Scotch-Weld DP405 epoxy managed to provide sufficient bond strength to completely detach the chitosan coating in both tensile and shear strength test. However, the surface roughness of the stud or coupon which the adhesive sticks to is equally important. It was discovered that by roughening the surface of the stud and aluminium coupon used in tensile and shear strength tests had managed to increase the bond strength between stud/coupon by more than 2 times.

Silane was successfully used by Bumgardner et al. to chemically bond the chitosan to titanium surface via solution cast method for higher bond strength. It was reported that the tensile bond strength of the silanated coating (1.5 – 1.8 MPa) was three times more than the non-silanated coating [1]. However, the strength reported by Bumgardner et al. cannot be compared with the tensile bond strength reported in this study because their titanium substrates (wet grounded with 80 grit SiC paper) had rougher surface compared to the surface roughness of titanium substrates (wet grounded with 1200 grit SiC paper) in this study. The substrate was wet grounded with finer grit SiC paper in this study because it was thought that silane would bond better if the surface of the titanium substrate is smoother. In the tensile and shear strength tests,
Silane was used to chemically bond some of the chitosan coatings to the titanium substrate to evaluate if silane would work under electrospray method. Unfortunately, no significant strength difference between silanated and non-silanated coating was noticed. It was thought that extended period of exposure of silane to high electric field and flow of electron compromised its effect. However, tensile bond strength of silanated and non-silanated coatings produced using solution cast method did not show significant strength difference as well. Fourier-transform infrared spectroscopy (FTIR) was done by Bumgardner et al. after the result from the tensile test was reported to them. Result from FTIR shows very little –OH group was developed on the surface of the titanium, this lead to silane not able to react and bond to the surface of the titanium substrate hence the insignificant difference between the bond strength. The failure of forming –OH group on the surface of titanium substrate after 24 hrs water bath was yet to be determined.

The result of the tensile bond strength test has shown that electrospray coatings were significantly stronger compared to solution cast coatings according to analysis of variance and Tukey’s post hoc test. This could probably due to the advantage of breaking the chitosan solution into size of nano to micro particles/droplets before depositing them layer by layer on the surface of the substrate using electrospray. The small sized droplets would be able to effectively fill the cavity or microcracks on the surface of the titanium hence increase the area of the bonding between the coating and substrate. This would be difficult for solution cast method to fill the cavity and microcracks since chitosan solution in bulk was poured directly on the surface of substrate. Besides that, the viscous chitosan solution would trap and prevent microbubble from leaving the cavity or microcracks on the surface of substrate. However, results from shear bond strength test didn’t indicate significant difference in bond strength between electrospray and
solution cast coatings. This might be due to the limited number of test samples (n = 5) done on shear bond strength test. More number of test samples should be done to verify the result.

Results from the tensile bond strength test also shows high variability in electrospray coatings compared to solution cast coatings. Since the electrospray coatings were built up in additive way, this may have induced cracks or poor bonding between each layer resulting in high variability in coating strength. Due to the limited flowrate from singled capillary electrospray, the first few layers of the coating were dry or partially dry deposited due to evaporation of the droplets before a liquid layer was formed. This would result in poorer bonding between each subsequence layer since dried or partially dried droplets will not bond properly together. Besides that, the electrospray was done in opened air ambient conditions. The surface of the substrate and coating may be contaminated by dust particle in the atmosphere. Furthermore, the humidity of the air would affect the rate of evaporation of the sprayed droplets contributing to inconsistent coating strength. The variability of surface roughness between the titanium substrates would play an important factor too. Although all titanium substrates were wet grounded with the same grit of SiC paper, the grounding was done by hand instead of a holder on a grinder/polisher. As a result, the applied force as well as the position of the force applied to the substrates on the grinder/polisher varied from one another. This could result to variability in surface roughness between substrate to substrate. Besides that, increasing the bonding area between the stud and coating by using a larger diameter stub would improve the sensitivity of the tensile bond strength of the coating.

The results collected from tensile and shear bond strength tests have shown that the bond strength of coatings produced using electrospray method was comparable to coatings produced by solution casting method. The tensile bond strength of the electrospray coatings was even
higher than the solution cast coating. This shows that electrospray is a viable deposition technique with good controllability on coating thickness. With a more standardized electrospray process such as a controlled environment with clean air and humidity control, the coatings produced could achieve stronger and better repeatability in strength.
3. Electrospray Deposition of Chitosan on 3D Surface

3.1. Determination of Test Parameters

The efficacy of coating chitosan using electrospray deposition method on 3D surface is evaluated by coating on titanium screw. In this study, chitosan coating produced using electrospray method on non-silanated titanium screw was conducted to study how well electrospray method can be used to coat 3D surface with irregular contour. Functional bone simulation test is conducted to evaluate the durability of the coating on the titanium screw. The test was done by inserting the coated screw into polyurethane bone density foam and the percentage of the coating mass loss was evaluated. The test parameters were referred from the dental implant surgical manual from Hahn and Biohorizons [39, 40]. According to the manuals, pilot hole is drilled into the jaw bone before the insertion of dental implant. The diameter of the pilot hole is 0.5 – 0.6 mm smaller than the diameter of the implant (screw). The titanium screw used for the simulation test has thread diameter of 4.72 mm (0.186 inch) and root diameter of 3.51 mm (0.138 inch). Based on the thread diameter of the screw, the pilot hole diameter should be 4.12 mm (4.72 mm – 0.6 mm). However, the pilot hole was drilled to the size of the root diameter of the screw because the diameter is smaller and will induce more stress to the coating. Furthermore, the implant is inserted at a low rotational speed of 30 rpm. The reason is to prevent excessive generation of heat causing thermal necrosis during real implant surgery.

3.2. Methodology

3.2.1. Materials

Titanium screws with dimension of #10 × 3/4 Pan Head, Philips drive (Allied Titanium, Part no.: 0001599) was selected as the 3D substrate for chitosan coating. The titanium screws came with unpolished finish surface. 92.6% de-acetylated chitosan powder (Heppe Medical
Chitosan GmbH, product no.: 24711) with molecular weight of 300 – 700 kDa was used to prepare the chitosan solution. Reagent alcohol (VWR Analytics, product no.: BDH1156-4LP) containing 95% ethanol + methanol and 5% 2-propanol solution was used to dilute the chitosan solution and improve its sprayability. Polyurethane bone density foam (General Plastics, LAST-A-FOAM FR-3708) with density of 128 kg/m³ was used to represent bone for implant insertion. Phosphate buffered saline solution (Fisher Scientific, BP399-1) was used to simulate the aqueous condition for functional bone simulation test.

3.2.2. Cleaning and Passivation of Titanium Screw

The titanium screws with unpolished finish surface were not subjected to any surface treatment technique to alter their surface roughness. The titanium screws were cleaned with alconox and warm water. Next, they were ultrasonically cleaned with acetone, ethanol and de-ionized water for 10 min each and passivated using 70:30 vol% de-ionized water/nitric acid for 30 mins at ambient conditions. The passivated screws were then rinsed with de-ionized water to remove the nitric acid. The cleaned and passivated screws were then dried at 110 °C for 10 mins to remove moisture on the surface of the screws.

3.2.3. Weighing and Storage of Titanium Screw

The individual mass of the screw (noted as \(m_{screw}\)) was measured using a mass balance. Each of the screw’s mass with its identifier were recorded. The screws were stored in a vacuum chamber and held in vertical position by sticking the screw head on adhesive tape. This is to prevent the screw from rotating freely in the vacuum chamber during transportation causing contamination to the threaded part of the screw as only the threaded part was to be coated. Air in the chamber was drawn out using a vacuum pump (Ted Pella, Value VRI-2 Rotary) to pressure range of 10 – 2 mbar. To depressurize the chamber, nitrogen gas or filtered air was flushed into
the chamber to prevent dust from depositing on the surface of the screw. These steps were repeated every time the vacuum chamber was opened.

3.2.4. Electrospray Process

The chitosan solution used for the electrospray process was prepared as described in chapter 2. B. v. Chitosan Solution Preparation. Similar electrospray setup was used to coat the screws as described in chapter 2. B. vi. Electrospray Process. However, a screw holder as shown in Fig. 20 below was assembled to hold and rotate the screw to coat the screw from all sides.

![Screw holder setup for electrospray process](image)

**Fig. 20.** Screw holder setup for electrospray process

The screw was mounted one side to the Phillips head screwdriver bits and the other to the soldered tip of the ground wire. The ground wire was inserted through plastic screw so that the titanium screw can be held in place by tightening the plastic screw. The screw holder was mounted on the translation stage to move the screw left and right relative to the position of the capillary. The stepper motor (Longruner, 28BYJ-48) was used to rotate the screw to coat the screw from all sides along the threaded section. A feedback system was created to control the
timing and angle of rotation of the stepper motor. The wiring schematic of the feedback system is as shown in Fig. 21 below. The program flowcharts of the translation stage controller and Arduino Uno microcontroller are as shown in Fig. 22.

![Wiring schematic of the feedback system](image)

**Fig. 21.** Wiring schematic of the feedback system
Fig. 22. Program flowcharts of translation stage and Arduino Uno microcontroller
Fig. 23. Spray position for each iteration illustrated as blue and red lines on the surface of the screw

A microcontroller (Arduino, Uno) was used as the processing unit in the feedback system to process input signal from the translation stage controller and output signal to the stepper motor to rotate the screw. To determine the timing to rotate the screw, the translation stage controller was programmed to toggle between 24 V and 0 V at ‘Output Port 1’ every time the translation stage completes its movement to the right or left. The output signal from the translation stage was stepped down to 5 V maximum using a voltage divider to be compliance with the input voltage of the microcontroller. The microcontroller was programmed to ‘listen’ for change in state of the input signal at ‘Digital pin 7’. Once a change in state of the input signal is detected, the microcontroller will check if the total angle of rotated stored in a counter is equal to $324^\circ$ ($360^\circ - 36^\circ$). If the logical test is false, it implies that the screw has yet to be coated in a full $360^\circ$ revolution. The screw will be rotated $36^\circ$ to the next position with similar color as shown in Fig. 23. The counter recording the rotated angle will be incremented by $36^\circ$. On the other hand, if the
test returns true, it implies the screw has made a full $360^\circ$ revolution. The screw will be rotated $18^\circ$ to the next position with different color as shown in Fig. 23. The counter recording the rotated angle will reset to zero. In other words, the spray position of the screw alternates between blue and red after every full $360^\circ$ revolution (blue $\rightarrow$ red $\rightarrow$ blue …). The reason is to maintain the liquid state of the sprayed chitosan solution by reducing the time it takes for the screw to rotate a full revolution. The stepper motor was controlled by the microcontroller through a drive module board (Longruner, ULN2003). The drive module board receives 4 digital input signals from the microcontroller and activates the stepper motor’s coils accordingly. The stepper motor was programmed to rotate at a constant angular velocity of 20 rpm. It would take 0.3 s to rotate $36^\circ$ at this angular velocity. Thus, the 0.3 s delay every time after the translation stage completed a movement. The stepper motor will continuously rotate if the state of input signal has changed more than 200 times. The continuous rotation of the screw is to prevent the sprayed liquid chitosan solution from continuously accumulate at the bottom side of the screw due to its weight and drip off from it. The continuous rotation of the screw also ensures the uniform thickness of coating around the screw.

The spray parameters used for coating screw are summarized in Table 6 below.

**Table 6**

Spray parameters for screw

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Output voltage of high voltage regulator</td>
<td>6.0 kV</td>
</tr>
<tr>
<td>Capillary tip to screw distance</td>
<td>3 mm</td>
</tr>
<tr>
<td>Capillary type, length and ID</td>
<td>Silica, 38 cm, 250 µm</td>
</tr>
<tr>
<td>Spray flowrate</td>
<td>18 µl/min</td>
</tr>
<tr>
<td>Spray time</td>
<td>42 mins</td>
</tr>
<tr>
<td>Spray volume per screw</td>
<td>0.75 ml</td>
</tr>
</tbody>
</table>
The speed of the translation stage was set at 12.2 mm/s. The coated titanium screws with liquid chitosan solution were removed together with the screw holder to a flat surface while the stepper motor rotates continuously until the coating is dried. Then, the coated screws are removed from the screw holder and neutralized before subjected to test.

3.2.5. Neutralization

The coated screws were neutralized to remove the acetic acid in the coating prior to the test. The screws were placed individually in a labeled small petri dish and suspended in 0.25 M phosphate buffer solution and placed on a belly dancer for 20 mins. The screws were then rinsed with de-ionized water and left for 24 hours to dry. The mass of the coated screws (noted as \( m_{\text{screw+coating}} \)) was weighted again before being tested.

3.2.6. Functional Bone Simulation Test

The functional bone simulation test tends to simulate the placement of the chitosan coated screw into bone. Polyurethane bone density foam with density of was used to represent bone for implant insertion. Pilot holes with diameter of 3.56 mm were drilled using drill bit for the coated screw insertion. The diameter of the pilot holes was slightly larger compared to root diameter (3.51 mm) of the screw. The drilled polyurethane bone density foam was cleaned using compressed air to remove the foam residual left after drilling. The polyurethane bone foam was then fully suspended in phosphate buffered saline solution diluted with deionized water to 10:90 vol% phosphate buffered saline/deionized water. The phosphate buffered saline was used to simulate the aqueous condition of the implant insertion procedure. The suspended polyurethane foam was placed under vacuum to draw out the air trapped inside the foam for an hour. The screws were inserted into the polyurethane foam using a power drill at constant rotational speed of 30 rpm. The screws were removed by cutting through the polyurethane foam. Precaution was
taken to ensure the remaining coating on the screw was not damaged during the cutting process.
The extracted screws were dried for 24 hrs and then cleaned with compressed air to remove any
attached polyurethane foam residual. The mass of the screws after test (noted as \( m_{\text{after test}} \) was
weighted. The percentage of the remaining coating can be calculated by the following equation:

\[
\text{Percentage of remaining coating (\%) } = \frac{m_{\text{after test}} - m_{\text{scREW}}}{m_{\text{coating+screw}} - m_{\text{scREW}}} \times 100
\]  

(1)
3.3. Results

3.3.1. Coating on Titanium Screw

Electrospray method has successfully used to coat the titanium screws. Despite the complex and irregular contour on the threaded portion of the screw, electrospray method has proven to be an effective method to deposit uniform chitosan coating on it. Fig. 24 below shows a coated and non-coated titanium screw.

![Fig. 24. Chitosan coated and non-coated titanium screw](image)

3.3.2. Functional Bone Simulation Test

The functional bone simulation test was done on 12 non-silanated chitosan coatings produced by electrospray method. Silanated coating was not tested because the effect of silane was not significant according to the tensile and shear bond strength test result. The tested screws
were examine using light microscopy to visually identify the coating worn locations. The remaining and worn location of the coating are shown in Fig. 25 below.

**Fig. 25.** Light microscopy of the screw after functional bone simulation test and non-coated screw as reference
It was observed that all the tested screws have coating removed at the first few threads of the screw. Furthermore, coating tends to wear off at the outer diameter of the thread. The percentage of mass loss is shown in Table 7 and Fig. 26 below.

**Table 7**

Mass of screw and coating before and after test

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mass of Screw, $m_{screw}$ (g)</th>
<th>Mass of Screw and Coating, $m_{screw+coating}$ (g)</th>
<th>Mass of Coating, $m_{coating} = m_{screw+coating} - m_{screw}$ (g)</th>
<th>Mass of Screw and Coating After Test, $m_{aftertest}$ (g)</th>
<th>Mass Loss, $m_{aftertest} - m_{screw+coating}$ (g)</th>
<th>Percentage Mass Loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5427</td>
<td>1.5517</td>
<td>0.0090</td>
<td>1.5499</td>
<td>0.0018</td>
<td>20.0000</td>
</tr>
<tr>
<td>2</td>
<td>1.5363</td>
<td>1.5471</td>
<td>0.0108</td>
<td>1.5455</td>
<td>0.0016</td>
<td>14.8148</td>
</tr>
<tr>
<td>3</td>
<td>1.5358</td>
<td>1.5449</td>
<td>0.0091</td>
<td>1.5439</td>
<td>0.0010</td>
<td>10.9890</td>
</tr>
<tr>
<td>4</td>
<td>1.5366</td>
<td>1.5458</td>
<td>0.0092</td>
<td>1.5444</td>
<td>0.0014</td>
<td>15.2174</td>
</tr>
<tr>
<td>5</td>
<td>1.5413</td>
<td>1.5507</td>
<td>0.0094</td>
<td>1.5494</td>
<td>0.0013</td>
<td>13.8298</td>
</tr>
<tr>
<td>6</td>
<td>1.5405</td>
<td>1.5500</td>
<td>0.0095</td>
<td>1.5487</td>
<td>0.0013</td>
<td>13.6842</td>
</tr>
<tr>
<td>7</td>
<td>1.5409</td>
<td>1.5516</td>
<td>0.0107</td>
<td>1.5508</td>
<td>0.0008</td>
<td>7.4766</td>
</tr>
<tr>
<td>8</td>
<td>1.5393</td>
<td>1.5503</td>
<td>0.0110</td>
<td>1.5493</td>
<td>0.0010</td>
<td>9.0909</td>
</tr>
<tr>
<td>9</td>
<td>1.5408</td>
<td>1.5525</td>
<td>0.0117</td>
<td>1.5515</td>
<td>0.0010</td>
<td>8.5470</td>
</tr>
<tr>
<td>10</td>
<td>1.5365</td>
<td>1.5476</td>
<td>0.0111</td>
<td>1.5461</td>
<td>0.0015</td>
<td>13.5135</td>
</tr>
<tr>
<td>11</td>
<td>1.5368</td>
<td>1.5471</td>
<td>0.0103</td>
<td>1.5458</td>
<td>0.0013</td>
<td>12.6214</td>
</tr>
<tr>
<td>12</td>
<td>1.5360</td>
<td>1.5469</td>
<td>0.0109</td>
<td>1.5457</td>
<td>0.0012</td>
<td>11.0092</td>
</tr>
</tbody>
</table>

Average mass of coating deposited (g): 0.0102
Std Dev: 0.0009

Average percentage mass loss: 12.5662
Std Dev: 3.4396
95% CI: 10.3808 – 16.0058
3.4. Discussion

Another aim of this study was to investigate the effectiveness of using electrospray to coat 3D surface. Conventional method which is solution casting is poor at coating 3D object with complex surfaces especially when the coating needs to be uniform in thickness. In this study, it was observed that electrospray was able to coat the titanium screw uniformly with ease despite the complex contour of the screw. Besides, the standard deviation of 0.9 mg of the weighted mass on each screw suggests that electrospray could precisely control the mass of chitosan coated on the screw. The coated screws were inserted into polyurethane bone density foam soaked with phosphate buffered saline solution to evaluate the retention of the coating. Only electrospray and non-silanated coating on screw was evaluated because it is difficult to control...
the same amount of chitosan deposited on electrospray coating using solution cast method. The functional bone simulation test resulted an average of 12% loss in mass of coating. With light microscopy, it was observed that majority of the coating was still intact on the screw as shown in Fig. 25. However, all tested screws showed sign of wear near the first few threads of the screw as well as the outer diameter of the thread. The wear at the first few threads of the screw was due to the higher number of turns it rotates in the polyurethane bone foam compared to the portion of the screw near the head. The longer the coating experience shearing, the less the coating retains. Furthermore, the wear at the outer diameter of the thread was due to higher level of shear stress at the tip of the thread caused by the compression of the bone foam. Since the pilot hole was not threaded prior to the insertion of the screw, the screw will experience higher level of shear stress and compression when inserted.

Another potential cause of coating wear was due to the nature of chitosan coating turning soft gel-like when it encounters water after a short period of time. This would reduce the bond strength of the coating to the substrate as well as increase the loss due to shearing. One potential way to circumvent this problem is by increasing the hydrophobicity of the coating to increase the time it takes to absorb water and turn soft. However, increasing the hydrophobicity of the coating might have detrimental effect to the cell adhesion. Furthermore, a secondary layer or sacrificial layer of chitosan coating can be dry deposited on the primary coating to protect it from wear and tear. Since the dry deposited chitosan layer bond loosely to the primary coating, it would wear gradually while serves as a cushion to reduce the shear stress on the primary coating. The additional thickness on the primary coating would prevent or delay water from coming into contact.
The effective performance of electrospray method on coating chitosan on 3D surface and its ability to precisely control the amount of chitosan deposited shows its potential use as delivery method for chitosan coating.
4. Recommendations

Both tensile and shear tests have shown no difference in strength between silanated and non-silanated coatings. FTIR results have shown the lack of –OH group forming on the surface of the titanium substrate hence the lack of silane bond to the surface. This could imply that the 24 hrs pure water bath was not effective in forming the –OH group. Alternative ways to enhance the formation of –OH group should be investigated.

The correlation of surface roughness of the titanium substrate and bond strength of chitosan coating should be investigated. It was observed that by roughening the surface of aluminium stud with 120 grit SiC paper for tensile test managed to increase the bond strength between the stud and epoxy by more than 2 times. This implies that surface roughness of the substrate could have significant effect on the bond strength of the coating.

One issue faced during the coating process was the variability of humidity and contamination from the surrounding environment. This would induce variability to the quality of the coating. This can be overcome by carrying out the coating process in a controlled environment such as an enclosed box with air filter and humidity control. Secondly, the throughput of the coating process was largely limited by singled capillary electrospray. The maximum achievable spray flowrate of the electrospray used in this study was 38 µl/min while maintaining reasonable spray stability. This can be overcome using multi-nozzles electrospray to increase the spray flowrate [36].

The disadvantage of wet deposition is the buildup of liquid layer on the surface of the substrate. This would be a problem for 3D surface since the liquid layer will be affected by gravity causing the accumulation at the lower part of the implant. One possible way to overcome
this is by spraying a more viscous liquid. This will require capillary with larger internal diameter since the pressure drop across capillary is affected by the viscosity of the liquid.

One disadvantage of chitosan coating is it turns soft gel-like when it encounters water after a short period of time. This would deteriorate the rigidity of the coating under moist condition. To overcome this issue, secondary/sacrificial layer of chitosan can be dry deposited on the primary layer to protect it. Since the dry deposited layer bonds weakly to the primary layer, it would gradually wear off when the screw is inserted. Besides, the extra thickness would delay the primary coating from getting wet before the screw is completely inserted.
5. Conclusions

The semi-autonomous electrospray system for coating 2D titanium substrate and titanium screw was developed and tested. The electrospray method is capable of coating 2D and 3D surfaces uniformly at ease. Tensile and shear tests have shown comparable bond strength of electrospray coatings to solution cast coatings. Besides, the tensile bond strength of electrospray coatings was statistically higher than solution cast coating. However, no significant strength difference was observed for silanated and non-silanated coatings. FTIR results show little –OH group was developed on the surface of titanium hence silane was not bonded to the surface. Results from functional bone screw simulation indicate an average of 12% loss in coating mass on the titanium screw after insertion. Major wear area is located at the first few threads of the screw and the outermost diameter of the thread. This was due to longer exposure to stress during insertion at the first few threads and higher level of stress at the outer diameter of the thread.
References


[6] Rajendra, PP, Sunil, UT, Suresh, US, Jalinder, TT and Abraham, JD, "Biomedical applications of poly(lactic acid)." Recent Patents on Regenerative Medicine, 4 (1) 40-51 (2014)


[23] Qin, JK, He, HY, Zhang, WJ, Chen, FP and Liu, CS, "Effective incorporation of rhbmp-2 on implantable titanium disks with microstructures by using electrostatic spraying deposition." Rsc Advances, 6 (57) 51914-51923 (2016)


Appendices

A. Coding For Translation Stage (2D Substrate)

```plaintext
#axis xyz;
#units inch;

#define y 1.4488192;
#define x 1.4488192;
#define yd 0.14488192;
#define xd 0.14488192;
#define spd 2000;
#define offy 0.07244096;
#define offx 0.07244096;
#define yminusyd 1.30393728;
#define xminusxd 1.30393728;
#define ydiv 5;
#define xdiv 5;
#define yddiv 4;
#define xddiv 4;
#define nlayer 14;
#define yoff 4.6488192;
#define zoff 5;

#reference xyz;

#input

moveabs 0(4000),0(4000),zoff(4000),0(4000);
wait 65;
moveabs 0(4000),0(4000),0(4000),0(4000);
wait 65;

pattern1:
    repeat
        move 0(spd),y(spd),0(spd),0(spd);
        move xd(spd),0(spd),0(spd),0(spd);
        move 0(spd),-y(spd),0(spd),0(spd);
```
move xd(spd),0(spd),0(spd),0(spd);
until xdiv;
move 0(spd),y(spd),0(spd),0(spd);
goto pattern2;

pattern2:
repeat
move -x(spd),0(spd),0(spd),0(spd);
move 0(spd),-yd(spd),0(spd),0(spd);
move x(spd),0(spd),0(spd),0(spd);
move 0(spd),-yd(spd),0(spd),0(spd);
until ydiv;
move -x(spd),0(spd),0(spd),0(spd);
goto pattern3;

pattern3:
move offx(spd),offy(spd),0(spd),0(spd); repeat
move 0(spd),yminusyd(spd),0(spd),0(spd);
move xd(spd),0(spd),0(spd),0(spd);
move 0(spd),-yminusyd(spd),0(spd),0(spd);
move xd(spd),0(spd),0(spd),0(spd);
until xddiv;
move 0(spd),yminusyd(spd),0(spd),0(spd);
move xd(spd),0(spd),0(spd),0(spd);
move 0(spd),-yminusyd(spd),0(spd),0(spd);
goto pattern4;

pattern4:
repeat
move -xminusxd(spd),0(spd),0(spd),0(spd);
move 0(spd),yd(spd),0(spd),0(spd);
move xminusxd(spd),0(spd),0(spd),0(spd);
move 0(spd),yd(spd),0(spd),0(spd);
until yddiv;
move -xminusxd(spd),0(spd),0(spd),0(spd);
move 0(spd), yd(spd), 0(spd), 0(spd);
move xminusxd(spd), 0(spd), 0(spd), 0(spd);
move offx(spd), offy(spd), 0(spd), 0(spd);
goto pattern5;

pattern5:
    repeat
    move 0(spd), -y(spd), 0(spd), 0(spd);
    move -xd(spd), 0(spd), 0(spd), 0(spd);
    move 0(spd), y(spd), 0(spd), 0(spd);
    move -xd(spd), 0(spd), 0(spd), 0(spd);
    until xdiv;
    move 0(spd), -y(spd), 0(spd), 0(spd);
goto pattern6;

pattern6:
    repeat
    move x(spd), 0(spd), 0(spd), 0(spd);
    move 0(spd), yd(spd), 0(spd), 0(spd);
    move -x(spd), 0(spd), 0(spd), 0(spd);
    move 0(spd), yd(spd), 0(spd), 0(spd);
    until ydiv;
    move x(spd), 0(spd), 0(spd), 0(spd);
goto pattern7;

pattern7:
    move -offx(spd), -offy(spd), 0(spd), 0(spd);
    repeat
    move 0(spd), -yminusyd(spd), 0(spd), 0(spd);
    move -xd(spd), 0(spd), 0(spd), 0(spd);
    move 0(spd), yminusyd(spd), 0(spd), 0(spd);
    move -xd(spd), 0(spd), 0(spd), 0(spd);
    until xddiv;
    move 0(spd), -yminusyd(spd), 0(spd), 0(spd);
    move -xd(spd), 0(spd), 0(spd), 0(spd);
    move 0(spd), yminusyd(spd), 0(spd), 0(spd);
goto pattern8;
pattern8:
    repeat
    move xminusxd(spd),0(spd),0(spd),0(spd);
    move 0(spd),-yd(spd),0(spd),0(spd);
    move -xminusxd(spd),0(spd),0(spd),0(spd);
    move 0(spd),-yd(spd),0(spd),0(spd);
    until yddiv;
    move xminusxd(spd),0(spd),0(spd),0(spd);
    move 0(spd),-yd(spd),0(spd),0(spd);
    move -xminusxd(spd),0(spd),0(spd),0(spd);
    move -offx(spd),-offy(spd),0(spd),0(spd);
    send 90;
    loop nlayer times pattern1;

    moveabs 0(4000),yoff(4000),0(4000),0(4000);
    moveabs 0(4000),yoff(4000),zoff(4000),0(4000);

    stop.
    #start
B. Coding For Translation Stage (3D Substrate)

#axis xyz;
#units inch;

#define y 0.75590592;
#define spd 2000;
#define num 800;
#define zoff 5;

#define reference xyz;

#input
moveabs 0(4000),0(4000),zoff(4000),0(4000);
wait 65;
moveabs 0(4000),0(4000),3(4000),0(4000);
wait 65;

set3d on;
repeat
move 0(spd),0.25196864(spd),0.09448824(spd),0(spd);
move 0(spd),y(spd),0(spd),0(spd);
set_port A1,1=1;
delay 3;
move 0(spd),-y(spd),0(spd),0(spd);
move 0(spd),-0.25196864(spd),-0.09448824(spd),0(spd);
set_port A1,1=0;
delay 3;
until num;
set3d off;

moveabs 0(4000),0(4000),3(4000),0(4000);
moveabs 0(4000),0(4000),zoff(4000),0(4000);

stop.
#start
C. Coding For Arduino Controller

```cpp
#include <CheapStepper.h>
#define angle 36

CheapStepper stepper (8,9,10,11);
float anglecount = 0;
int Din = 7;
boolean Laststate = false;
float laststep = 360 - angle;
int countswitch = 0;

void setup() {
  stepper.setRpm(20);
  pinMode (Din,INPUT);
}

void loop() {

  if (digitalRead(Din) != Laststate) {
    Laststate = !Laststate;
    countswitch = countswitch + 1;
    if (anglecount < laststep) {
      stepper.moveDegreesCW (angle);
      anglecount = anglecount + angle;
    } else {
      stepper.moveDegreesCW (angle/2);
      anglecount = 0;
    }
  }
  while (countswitch == 200) {
    stepper.moveDegreesCW (360);
  }
}
```