DEVELOPMENT OF INJECTABLE BIOCERAMIC DRUG DELIVERY SYSTEM FOR SOLID TUMOR TREATMENT

by

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ABSTRACT

JAMES ANDREW HAIG. Development of injectable bioceramic drug delivery system for solid tumor treatment
. (Under the direction of DR. AHMED EL-GHANNAM)

According to the national cancer institute “In 2016, an estimated 1,685,210 new cases of cancer will be diagnosed in the United States and 595,690 people will die from the disease” and “The number of people living beyond a cancer diagnosis reached nearly 14.5 million in 2014 and is expected to rise to almost 19 million by 2024.” The usual treatment for cancer involves surgery, radiation, and chemotherapy. In an effort to reduce the side effects of chemotherapy while increasing its effectiveness, biomaterials are investigated as sustained drug delivery systems for targeted release. Recent studies have demonstrated the ability of custom made bioceramics to provide therapeutic doses of anticancer drug that eradicated tumor cells in vitro and in animal models. The objective of the research work in the present master’s thesis was to develop an injectable formula of bioceramic drug delivery system that can be injected directly into solid tumors. The drug release kinetics from the injectable ceramic was measured and its efficacy has been confirmed.

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SCPC	Silica-calcium phosphate composite
Cis	Cisplatin
HAP	Hydroxyapatite
BMP	Bone morphogenetic protein
XRD	X-ray diffraction
SEM	Scanning electron microscope
PMMA	Polymethylmethacrylate
5-FU	5-fluorouracil
α-TCP	α-tricalcium phosphate

CHAPTER 1: INTRODUCTION

According to the national cancer institute “In 2016, an estimated 1,685,210 new cases of cancer will be diagnosed in the United States and 595,690 people will die from the disease” and “The number of people living beyond a cancer diagnosis reached nearly 14.5 million in 2014 and is expected to rise to almost 19 million by 2024.” Many of these survivors suffer not only the effects of their cancers, but also the side effects of the treatments which saved their lives.

1.1 Chemotherapy and Side Effects

1.1.1 Side Effects of Systemic administration of Chemotherapies

Many common and effective chemotherapies cause short term as well as life altering long term side effects. According to the National Cancer Institute these including anemia, bleeding and bruising, delirium, edema, fatigue, hair loss, infection and neutropenia, memory or concentration problems, nausea and vomiting, pain, fertility problems, skin and nail changes, as well as many more[19]. There is much research being done to create new and more effective drugs and treatments with decreased side effects [20, 21, and 22].

However, the systemic drug administration would continue to result in side effects due to circulation of the drug in the blood.

1.1.2 Cisplatin

Cisplatin (Cis) is a chemotherapy found on the World Health Organization's list of essential medicines. Cis is an effective therapeutic agent for the treatment of many solid tumors. Administered systemically it causes significant side effects, including nephrotoxicity (kidney damage), neurotoxicity (nerve damage), nausea and vomiting, as well as ototoxicity (hearing damage).

1.2 Drug Delivery Systems and Controlled Release

An ideal drug delivery system would provide a controlled dose of drug during a time period appropriate for the treatment of its target, while not modifying the biological activity of the drug molecule [1]. The drug delivery system should also minimize the acute and chronic side effects of the treatment when compared to traditional systemic administration. There is also the potential advantage of a decrease in the amount of drug used as well as a decrease in the amount of doses necessary for treatment to be effective.

The origins of the controlled release of active agents can be traced back to the 1960's [2]. Since then, there has been research into strategies from macroscopic devices with constant drug release rates to nanoparticle systems with targeted or site controlled delivery of therapeutics [2]. Despite the many advancements in controlled release of drugs there are many more avenues left to be explored.

1.3 Biomaterials

Significant research effort has been directed to the use of biomaterials as a delivery system for drugs [30, 31, 32, 33]. The advantage of this method is high efficacy and minimal or no adverse side effects.

There are a variety of materials used in drug delivery systems; these include polymers, glasses, ceramics, and composites [1, 7, 8 13]. Bioceramics are currently being studied as a viable drug delivery system due to their longer duration of controlled drug release [3, 4, 5, 6, and 7]. At the same time different gels are being explored as a drug delivery system as well [8, 9, 10, 11, and 12].

There are several important criteria for a biomaterial to be considered as a drug delivery system. The first is biocompatibility, whether or not the body has a local or systemic response to the introduction of the materials. Another criterion is that there are no side effects from the degradation or dissolution products of the material. It is also necessary that the material be able to bind drug molecules and release them in a controlled fashion. It is essential that the material does not compromise the efficacy of the drug molecule. There are also economic and production factors; the drug should be easy to manufacture and sterilize.

1.4 Silica-Calcium Phosphate composites

Silica-Calcium Phosphate composites (SCPC) are a family of ceramic composites composed of modified silica and calcium phosphate minerals with varying ratios of silica and calcium components [14]. SCPC is a resorbable biocompatible material which makes it an important material for research. Past studies of SCPC have shown it to be

highly porous with a large surface area available for drug binding. The method for making and sterilizing SCPC is also very economical and simple.

SCPC has been studied in the past as a delivery system for both anticancer drugs and antibiotics [15, 16, and 17]. SCPC has also been used as a scaffolding material and a carrier for bone morphogenetic protein (BMP) and bone marrow for bone regrowth [14]. The following section will cover some of the ground work that led up to this project.

1.4.1 Advanced bioceramic composite for bone tissue engineering: Design principles and structure-bioactivity releationship [14]

Published in April of 2004, this article introduced SCPC as a novel material suitable for tissue engineering scaffolds or cell and drug delivery. The manufacturing method was described and proves to be a very simple and inexpensive process using a ball mill and sintering furnace. The ability of SCPC to adsorb proteins effectively was demonstrated in this study. Rabbit models were used to prove that SCPC can successfully be used to stimulate rapid bone generation. Corrosion analysis was also performed to explore how the material would react in biological environments.

This study included extensive research into the phases and composition of the materials. Differential thermal analysis was performed to determine the temperature of crystallization and phase transformations. The effect of heat treatment and chemical composition on phase transformation was also analyzed. X-ray diffraction (XRD) techniques were used to determine the crystalline phases formed in the SCPC across varying heat treatments and chemical compositions. The materials were also analyzed for their morphology and porosity using the scanning electron microscope (SEM). The porosity was also determined using a mercury intrusion technique.

The study showed SCPC to be a porous material made up of multiple phases of calcium and silicon minerals. A schematic of the makeup of SCPC can be seen in Figure 1 below. It shows the interconnected porous structure found in the material as well as the interconnected phases of the minerals.

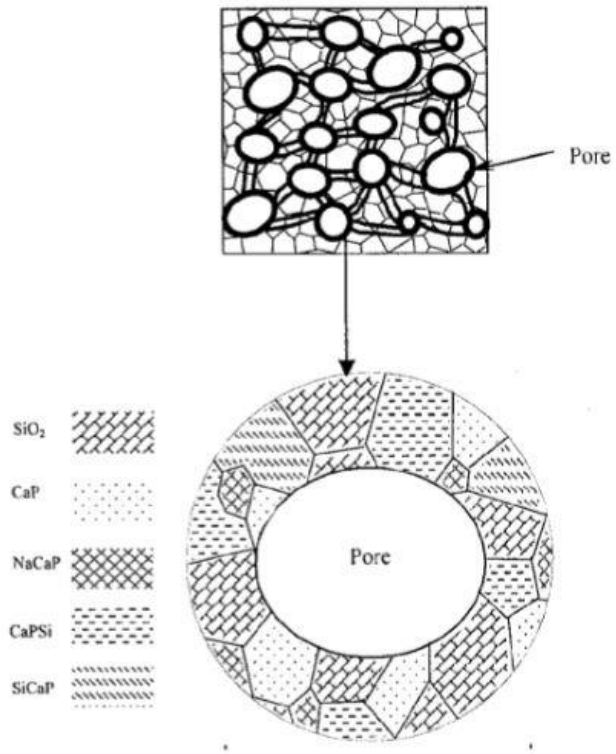

Figure 1: Schematic Cross Section of SCPC [14]

1.4.2 Cyclosilicate nanocomposite: A novel resorbable bioactive tissue engineering scaffold for BMP and bone-marrow delivery [23]

This article, published in 2004, shows how SCPC can be used effectively as carrier for BMP and a scaffold for tissue engineering. In this work, SCPC loaded with BMP was

able to enhance bone marrow cell differentiation and bone like tissue formation. The author notes that SCPC loaded with BMP may provide an alternative to autologous bone grafting.

1.4.3 Bone engineering of the rabbit ulna [18]

Published in August of 2007, this article describes the findings of a study performed to test the efficacy of SCPC loaded with bone morphogenetic protein (BMP) in stimulating bone growth in vivo. The study compared SCPC loaded with BMP to hydroxyapatite (HAP) loaded with BMP. The results comparing the release of BMP from SCPC vs HAP, seen in Figure 2, show that SCPC had a more advantageous release profile. The in vivo study portion of the paper showed that SCPC was excellent for not only acting as a carrier for BMP, but facilitating the growth of new bone.

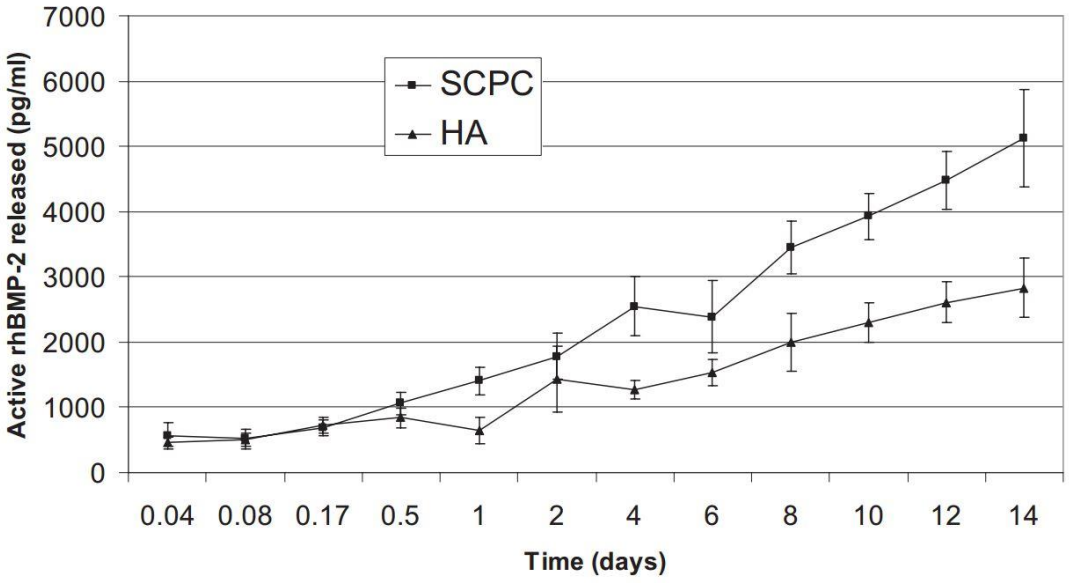

Figure 2: Release of BMP from SCPC and HAP [18]

1.4.4 Resorbable bioactive ceramic for treatment of bone infection [24]

Published in 2010, this paper discusses the results of a study to compare polymethylmethacrylate (PMMA) and SCPC when used as a carrier for antibiotics to treat osteomyelitis, a difficult to treat bone infection. The resulting data comparing the adsorption showed that the amount of antibiotic adsorbed by PMMA was more than 50% lower than that adsorbed by the SCPC. The release profile showed a greater sustained release profile from the SCPC as opposed to the PMMA. This paper showed the ability of SCPC to act as a drug delivery system for vancomycin.

1.4.5 A ceramic-based anticancer drug delivery system to treat breast cancer [17]

Published in July of 2010, this article showed that when loaded with 5-fluorouracil (5-FU) SCPC can be used as an effective treatment for solid tumors, while minimizing side effects. The study compared the release kinetics of two formulations of SCPC and both showed a favorable release profile. The efficacy of the drug after being released from the SCPC was also tested on 4T1 mammary murine tumor cells in vitro. The 5-FU proved to still be a potent anticancer agent after being released from the SCPC.

1.4.6 Evaluation of a bioresobable drug delivery system for the treatment of hepatocellular carcinoma [24]

This article, published in 2010, describes the results of a study where SCPC loaded with CIS was used to treat hepatocellular carcinoma (HCC). Hepatocellular carcinoma is a difficult to treat cancer also known generally as liver cancer, due to the fact that it originates in the liver. This article compares the release kinetics of SCPC50 and SCPC75, with SCPC75 having a significantly higher sustained release than the SCPC50. Table 1 shows the chemical composition of SCPC50 and SCPC75. During this study,

mercury porosimetry was also performed in order to determine the porosity of the materials, the results of which can be seen in Table 2. The in vitro study of CIS release from the SCPC samples showed that SCPC75 released a greater amount when compared to the SCPC50. This makes sence after seeing the porosity and surface area data on the two compositions of SCPC. A preliminary study was also performed to determine the cytotoxicity profile on H4IIE HCC cells in vitro. The results of this study showed that the CIS was still cytotoxic after being released from SCPC. Overall the study showed a lot of promise for SCPC as a drug delivery method.

Table 1: Composition of SCPC50 and SCPC75 [24]

Sample	SiO_2 (%)	P_2O_5 (%)	CaO (%)	Na_2O (%)	Particle Size (μm)
SCPC50	19.49	20.34	40.68	19.49	250–425
SCPC75	32.9	11.4	22.8	32.9	250-425

Table 2: SCPC50 and SCPC75 Porosity [24]

Sample	Total Pore Area (m^2/g)	Bulk Density (g/mL)	Total Porosity (%)
SCPC50	3.120	1.0650	53.93
SCPC75	18.299	0.5813	70.32

1.4.7 Engineering bioceramic microstructure for customized drug delivery

One of the successful strategies for using SCPC as an anticancer drug delivery methods performed previously by our group was using SCPC disks loaded with Cisplatin (CIS) and implanting them in the Hepatocellular carcinoma tumor sacks of male ACI rats [15]. The study showed that when compared to controls, the CIS loaded SCPC disks effectively delivered a therapeutic dose to its target and decreased the tumor size more effectively than conventional treatment. When compared to traditional systemic delivery the CIS loaded SCPC disks had an effect on organ toxicity very similar to the untreated animals, while the animals receiving systemic delivery of CIS had markedly higher levels of organ toxicity. Analysis of blood serum was performed to check for platinum content. The data showed that there was no demonstrable difference between the serum platinum in the CIS loaded SCPC animals and the control animals, while the animals who received systemic administration had significantly increased levels of platinum in the serum. Analysis of the tumor also showed a similar platinum concentration when comparing the CIS loaded SCPC implanted next to the tumor with systemic administration. The analysis also showed a significantly higher concentration of platinum in the tumors where the CIS loaded SCPC disks was implanted into the tumor.

When implanted near malignant tumors in animal models a more than 70% reduction in tumor volume was observed. The results of this study showed that SCPC can be used to deliver anticancer drugs in high concentrations to a tumor without having to expose the entire body to the drug dose. It also showed that the dissolution of the SCPC material itself did not cause any ill effects in the animal models.

1.4.8 Novel Bioceramic Urethral Bulking Agents Elicit Improved Host Tissue Responses in a Rat Model [25]

Published in July of 2016, this article describes the use of SCPC10 and crystobilite at urethral bulking agents and compares them to other bulking agents currently available on the market. The author used hyaluronic acid sodium salt, sodium hyaluronate (HA), to facilitate the injection of the particles. The author noted that the HA was rapidly resorbed into the body leaving the injected particles behind. For this study rat models were used to determine the efficacy as well as the physiological response of the two bioceramics. In this study, it was noted that there was no evidence of particle migration found. This is important because past studies have shown that smaller particles can migrate to distant organs. It was found that comparable levels of Ca, Na, and P ions were noted in distant organs of the experimental animals when compared to control animals. The animals with SCPC10 showed similar Si levels to the control animals, while those who received crystobilite or Macroplastique (a market product) had higher concentrations of Si than the controls. It is also important to note, in this study immunostaining of cross sections of the rat urethra did not reveal active macrophages or scarring for the SCPC10 samples. This suggests that there was little or no foreign body response to the SCPC10.

1.4.9 Summary of Above Studies on SCPC

The preceding studies show that SCPC is an effective material for use as a drug delivery system. These studies show that SCPC has been successful in delivering drugs in vivo, as well as delivering proteins and spurring bone growth. Most of these studies have necessitated an implantation or some type of surgical intervention to apply the delivery system to the affected area.

1.5 Studies of Note

The following are recent studies that provide a small glimpse into some of the work being done in the area of bioceramic drug delivery.

1.5.1 Controlled Release of Chemotherapeutic Platinum-Bisphosphonate Complexes from Injectable Calcium Phosphate Cements (CPC) [3]

In this article from 2016, Farbod et al describe a method of loading an α-tricalcium phosphate (α-TCP) cement and hydroxyapatite nanoparticles with a platinum anticancer drug (platinum-bisphosphonate complexes). Platinum-bisphosphonate complexes are a family of platinum based chemotherapeutic agents that have become a focus for researchers looking to treat bone malignancies [21]. This study showed that drug-loaded CPCs were chemotherapeutically active and through different treatments the activity of the chemotherapeutic agent could be modified. The research showed that not only could a steady rate of drug release be achieved from the drug loaded CPC, but that the amount of drug loaded and the release profile could be modified by using PLGA microspheres.

1.5.2 Hydroxyapatite crystals as a local delivery system for Cisplatin: adsorption and release of Cisplatin in vitro [5]

In this article Barroug and Glimcher describes the results of adsorption and release of Cisplatin from slurries of hydroxyapatite crystals. The authors tested the adsorption of Cisplatin in phosphate buffer solution (7.4 pH), phosphate buffer saline, and tris buffer. The authors found that in the presence of phosphate ions are essential for the adsorption of CIS to HAP crystals, while chloride ions inhibit this adsorption. The authors also found that chloride ions accelerated the release of CIS from the HAP crystals. The authors end by noting that they have done preliminary studies on rat osteosarcoma cells

and have found Cisplatin present in sufficient concentrations to kill the cells. In this research it is of note that 58% of the CIS was released after 2 weeks.

1.5.3 Apatite cement containing Cis-diamminedichloroplatinum implanted in a rabbit femur for sustained release of the anticancer drug and bone formation [4]

In this paper Tahara and Yoshiaki describes a method of loading apatite cement with platinum anticancer drug and implanting it into a rabbit femur for the purpose of treating cancer and replacing resected bone. An in vitro test was run to determine the release profile of the CIS from the apatite cement. An in vivo experiment was also done using rabbits.

The in vitro test was performed with 3 ratios of CIS loaded apatite cement: 5%. 10%, and 20%. The results showed that the 5% released the least, the 10% released the second most, and the 20% released the most platinum. This is to be expected, as each contained more platinum than the previous sample.

The purpose of the in vivo experiment was to determine the Pt concentration in bone marrow, kidney, and liver after implantation with the CIS loaded apatite cement. For the in vivo experiment there was a group that received systemic administration of CIS, then 4 groups received implants with varying amounts of CIS loaded apatite content in the order of 0%, 5%, 10%, and 20%. Upon inspection of the platinum content in the kidney and liver it was found that the implant groups had a higher concentration of platinum in these organs than the systemic group, with the 20% having the most and 5% the least. The histology at the the sight of the implant showed excellent bone union in all but the 20% samples. Ultimately the authors concluded that the 10% samples would make the ideal

implant, as they balanced the ability to form bone, release platinum, and minimize effects on organs.

1.6 Objectives

In most of the before mentioned studies, the bioceramics are administered through a surgical intervention. The purpose of this study is to investigate injectable bioceramic delivery systems in order to sidestep the complications and hazards associated with surgical implantation.

The first objective of this study is to formulate an injectable ceramic drug delivery system. In order to determine the optimal formulation several variables were considered. These included ceramic particle size, ceramic particle concentration, type of gel, gel concentration, and needle size. It was also necessary to determine a method to grade the performance of each formula. Sodium hyaluronate and alginate were chosen as the gels due to their biocompatibility and use in similar studies [25, 26, 27, and 28].

The second objective was to determine the effect of the gel on the release kinetics of the Cis loaded ceramic. This was necessary to ensure that the drug still released from the ceramic in a predictable and repeatable way.

Chapter 2: MATERIALS AND METHODS

2.1 Gel Preperation

2.1.1 Alginate Gel

The alginate gel was prepared by using alginic acid sodium salt from brown algae (Sigma, catalogue #A2033) mixed with nano-purified water (18.2 MΩ/cm). Three different alginate gel concentrations were created; 2%, 3%, and 4% weight/ volume. Table 3 shows the alginate weight and water volume used for each concentration. The alginic acid sodium salt samples were dissolved in purified water in a beaker with a magnetic stirrer and mixed for 4hrs to prepare completely homogeneous solutions of concentrations 2%, 2.5%, and 3%. Alginate solution at a concentration 4% became too thick in the beginning of mixing and therefore had to be mixed by hand with a spatula.

Table 3: Weight and Water Volume Used for Alginate Gel

	2%	2.5%	3%	4%
Alginate Weight (g)	3.004	2.5004	4.5074	20.0447
Water Volume (mL)	150	100	150	500

2.1.2 Hyaluronate Gel

The hyaluronate gel was prepared by using sodium hyaluronate (Novozymes, Tianjin, China) which was mixed with nano-purified water (18.2 MΩ/cm). A mass-volume percent of 2.5% was used for the hyaluronate gel as reported by Mann-Gow et al [28]. For the injectability test, a total of 377.1 mg of sodium hyaluronate was combined with 15mL of nano-pure water and then mixed in a small beaker with a magnetic stirrer for 12 hours. For the drug release kinetics, a separate batch hyaluronate gel was prepared using 2 grams of sodium hyaluronate in 80mL of nano-purified water and mixed for 12 hours.

2.2 Bioceramic Carrier Preparations

SCPC50 and SCPC75 were prepared as described in an earlier publication [15]. The Hydroxyapatite was made using bovine bone heated in a furnace to remove the organic material. All of the ceramics were ground using an agate mortar and pestle, sifted using a stack of ASTM sieves of a decreasing mesh size mounted on a W.S. Tyler RX-29 test sieve shaker and allowed to operate for 2 minutes at a time. Two particle size ranges were selected for the injectability test; 90μm-150μm and 150μm-250μm.

2.3 Injectability Test

The injectability of the ceramic-gel mixture was evaluated for several combinations of variables including: two gel types (Alginate and Hyaluronic acid), 4 gel concentrations (2, 3 and 4% alginate and 2.5 % hyaluronic), two different ceramic concentrations (20% and 40%), three different needle sizes (16, 18 and 20 G). Each ceramic was combined with the gel using a vortex for 1 min. The gel-ceramic mixture was drawn into a 1ml syringe and then injected in air and scored for difficulty of injection and flow.

The ease of injectability of the ceramic-gel mixtures was scored adopting scoring method modified from that devised by Cilurzo et al [29]. Table 4 demonstrates the scores for the injectability test.

Table 4: Injectability Scoring System

1	Injection: Not possible or very difficult
	Flow: No flow
2	Injection: Difficult
	Flow: Dropwise
3	Injection: Moderate
	Flow: Dropwise then continuous
4	Injection: Easy
	Flow: Continuous

2.4 Drug Loading on Bioceramic Carrier

A Cisplatin (Cis) solution of 10mg/ml in dimethyl sulfoxide (DMSO) was prepared by combining 1.0096 gm of Cis with 100ml of DMSO and mixing using a magnetic stirrer

for 48hr. To load the ceramic with Cis, 6 grams of each ceramic were placed in 18ml of Cis/DMSO solution in a 50ml polyethylene tube and placed in an incubator at 37°C for 48 hours. The bulk of the solution was then pipetted off and the ceramic was removed and allowed to dry in the air under laminar flow hood.

To determine the amount of drug adsorbed on the ceramic, 200 mg of ceramic-drug hybrid (n =3) were placed separately in 5 ml of 70% nitric acid and placed in an orbital shaker at a speed of 120 rpm for 120 hours, this work was completed under the fume hood. The Pt concentration in the nitric acid solution was measured using ICP-OES and taken as the Cis Concentration.

2.5 Measurement of Cisplatin Concentration by ICP OES

In order to measure the concentration of released elements a Perkin Elmer Optima 2100DV inductively coupled plasma optical emission spectrometer (ICP-OES) was used. The bio-ceramics used are constituted of calcium, silicon, phosphorus, and sodium, while the drug used had a main component of platinum, therefore these five elements were measured. Standard solution for each element was prepared, measured on the ICP-OES and the concentrations were used to construct standard curves for each element (Figure 3). The formulae derived from these standard curves were then used to calculate the concentration of an element in an unknown solution sample.

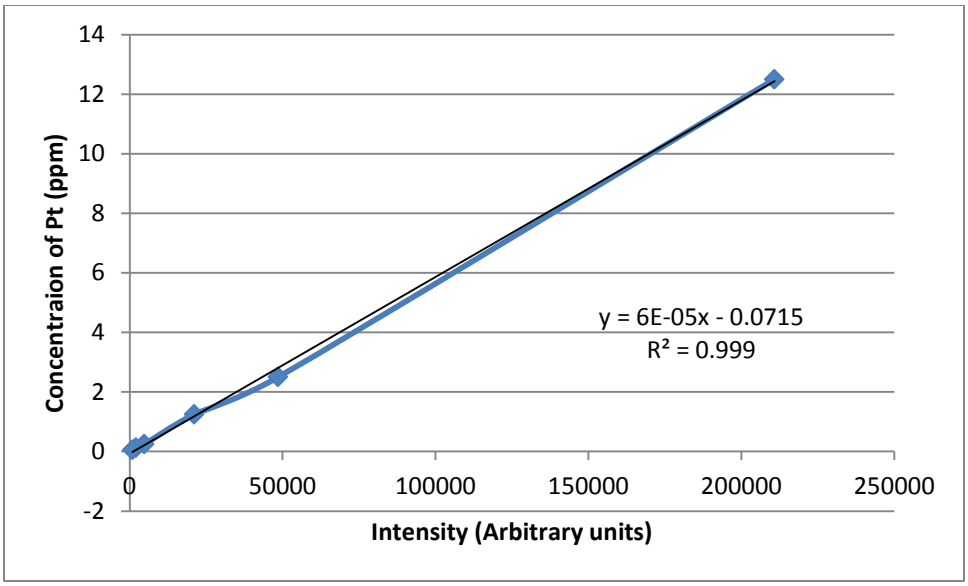

Figure 3: A Representative Standard Curve for Pt

Standard solutions for platinum were prepared from TraceCERT® platinum standard for ICP (sigma, catalogue #19078) with a starting concentration of 1000mg/L. From this standard, concentrations of 250, 125, 25, 12.5, 2.5, 1.25, 0.25, 0.125, and 0.05 mg/L were prepared. Standard solutions for calcium were prepared from TraceCERT® calcium standard for ICP (sigma, catalogue #94458) with a starting concentration of 10000mg/L. From this standard, concentrations of 10, 2, 1, 0.2, and0.1 mg/L were prepared. Standard solutions for silicon were prepared from TraceCERT® silicon standard for ICP (sigma, catalogue #08729) with a starting concentration of 10000mg/L. From this standard, concentrations of 200, 100, 20, 10, and 2 mg/L were prepared. Standard solutions for phosphorus were prepared from TraceCERT® phosphorus standard for ICP (sigma, catalogue #19916) with a starting concentration of 10000mg/L. From this standard, concentrations of 100, 50, 25, 10, and 5 mg/L were prepared. Standard solutions for

sodium were prepared from TraceCERT® sodium standard for ICP (sigma, catalogue #39924) with a starting concentration of 10000mg/L. From this standard, concentrations of 1000, 500, 250, 50, and 25 mg/L were prepared.

The ICP-OES was used in radial plasma view and the wavelengths used for each element are shown in Table 5.

Table 5: Wavelengths Used for ICP-OES Analysis

Element	Wavelength (nm)
Platinum	265.945
Calcium	315.887
Silicon	251.611
Phosphorus	213.617
Sodium	330.237

2.6 Drug Release Kinetics

1.1 gm of drug loaded and unloaded ceramic was mixed with 5.5 ml of each gel separately. The gel and ceramic combinations were then vortexed for 1 minute in 50 ml tube. Five (1ml) replicates (n=5) of the gel-ceramic mix were withdrawn immediately using 5 separate 1 ml “BD luer lock” syringes mounted with 16G needle. The ceramic-gel mixture was injected in 50ml test tubes containing 10ml of phosphate buffer solution (PBS), pH of 7.4. The tubes were tightly capped to avoid evaporation during incubation at 37 C in the incubator. Control experiments using ceramics loaded with Cis but not mixed with a gel were run in parallel. The tubes were then placed in an incubator at 37°C on an orbital shaker set to a speed of 120 rpm. At regular time intervals 1ml of the

solution was removed with a pipette and replaced with a fresh 1ml PBS to keep the volume constant. The time points, in hours, are: 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 264, 336, and 528. The concentration of Cis and ionic dissolution of the ceramic released was measured as mentioned above using ICP-OES.

Chapter 3: Results

3.1 Injectability

3.1.1 Scoring the Injectability

Building off of the work of Krhut et al, 2 ceramic particle size ranges were chosen, 90μm -150μm and 150μm -250μm. 2 types of gel were explored, hyaluronate and alginate, with varying concentrations. The alginate was tested at 2%, 3% and 4% w/v concentrations while the hyaluronate was tested at 2.5% w/v. 3 needle sizes were chosen:16G, 18G, and 20G.

Table 6: Scoring of Injectability

1	Injection: Not possible or very difficult
	Flow: No flow
2	Injection: Difficult
	Flow: Dropwise
3	Injection: Moderate
	Flow: Dropwise then continuous
4	Injection: Easy
	Flow: Continuous

The results, seen in Table 7, show that the 2.5% hyaluronate and the 2% alginate performed well through all needle sizes, while the 3% and 4% performed poorly in all but the largest needle size. The 40% ceramic concentration struggled with the thinnest gel and larger needles, therefore it was only tested in the formulation seen in the last column of Table 7. It is also worth noticing that the thicker alginate gels performed poorly through all but the largest needles, as did the larger particle size range. Figure 4 shows the ceramic particles suspended in hyaluronate after being injected.

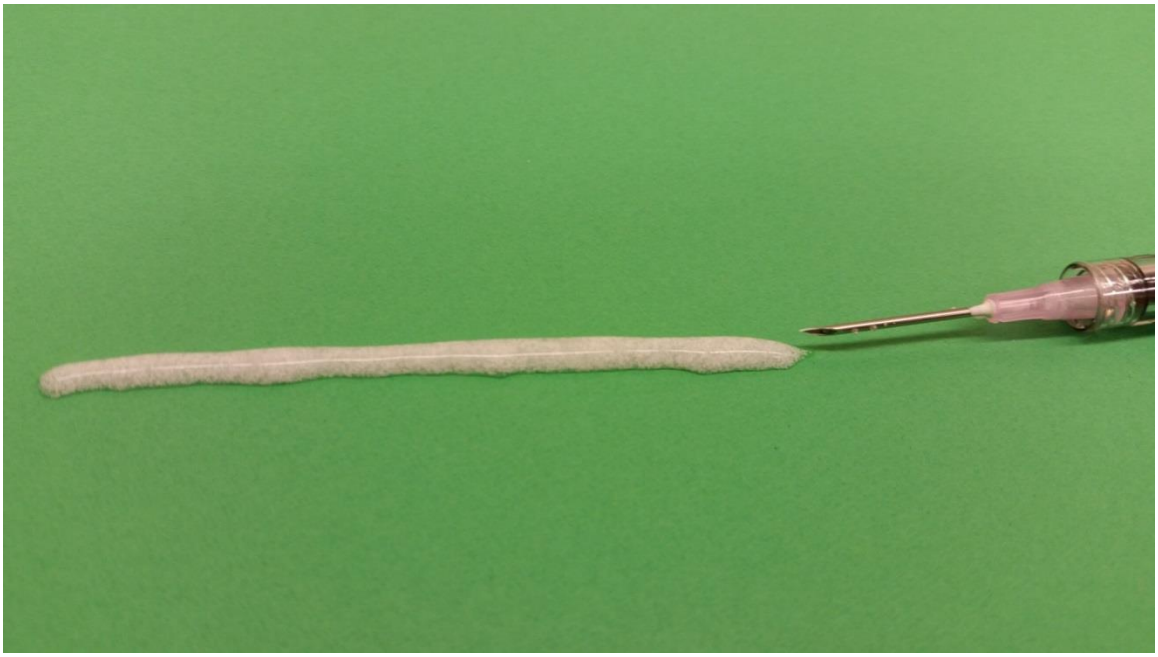

Figure 4: Injected Particles Suspended in Hyaluronate

Table 7: Scores of the Injectability of SCPC75 Particles of Different Size Ranges Suspended in Hyaluronate and Alginate Gels Using Different Needle Gauge Sizes

Needle Gauge	2.5% Hyaluronate 20% 90µm-150µm	2% Alginate 20% 90µm-150µm	3% Alginate 20% 90µm-150µm	4% Alginate 20% 90µm-150µm	2% Alginate 20% 150µm-250µm	3% Alginate 20% 150µm-250µm	4% Alginate 20% 150µm-250µm	2% Alginate 40% 90µm-150µm
16G injection	4	4	4	4	4	3	2	4
16G flow	4	4	4	3	4	4	2	4
18G injection	4	4	3	1	1	1	1	3
18G flow	4	4	4	1	1	1	1	3
20G injection	4	4	1	1	1	1	1	1
20G flow	3	4	2	1	1	1	1	1

3.2 Cisplatin Loading of Ceramics

The results of the drug loading measurements for the different ceramics can be seen in Figure 5. These results show that the SCPC75 has the highest concentration of Cisplatin loaded which agrees with past studies showing its higher surface activity and increased porosity [24]. The second highest concentration belongs to the SCPC50, while the lowest concentration belongs to the hydroxyapatite particles. This is to be expected due to the fact that hydroxyapatite is a very stable material with low surface activity and low porosity compared with the SCPC ceramics tested here. The difference between the SCPC75 and the HAP were found to be statistically significant. Past studies have shown there to be a difference in the loading capacities of SCPC75 and SCPC50, but the difference shown here is not statistically significant, the same with the SCPC50 and HAP.

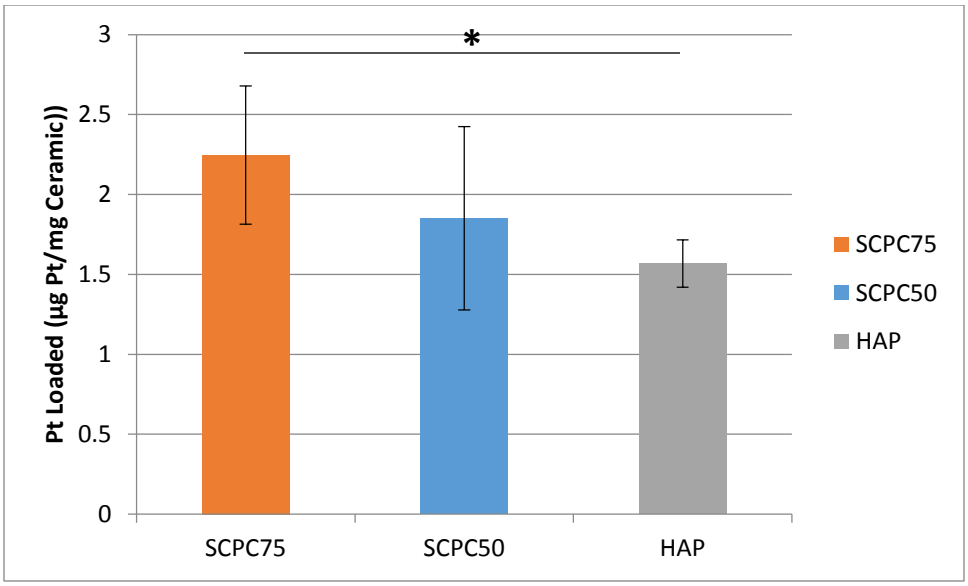

Figure 5: Cisplatin Concentration Loaded on Each Ceramic Type. *p<0.05

3.3 Drug Release Kinetics from Ceramic-gel Suspension

In order to determine the initial burst release the amount of drug released at the first 2 hours was measured and shown in Figure 6. The burst release graph shows the following:

1. Both alginate and Hyaluronate gels reduced the amount of released drug from all types of tested ceramics.
2. Suspending SCPC 50 particles in alginate and hyaluronate reduced the amount of drug released during the burst by 56% and 55 % compared to naked ceramic without gel, respectively. Statical anlysis showed that difference in the amount of drug released from SCPC50-alg and SCPC50-Hyal was not statistically significant.
3. Suspending SCPC 75 particles in alginate and hyaluronate reduced the amount of drug released during the burst by 41% and 73% compared to naked ceramic without gel,respectively. Statical anlysis showed that difference in the amount of drug released from SCPC75-alg and SCPC75-Hyal was statistically significant and showed a 54% difference.
4. Suspending HA particles in hyaluronate reduced the amount of drug released by 78% compared to naked ceramic without gel. The alginate gel could not be tested with HA due to crosslinking which occurs in alginate when mixed with HA.
5. Comparing the SCPC75 to SCPC50, the SCPC75 released significantly more than the SCPC50 in each category. In alginate the SCPC75 release was 80% greater than the SCPC50. In Hyaluronate the SCPC75 was 57% greater than the

SCPC50. For the naked particles the SCPC75 burst release was 74% greater than the SCPC50

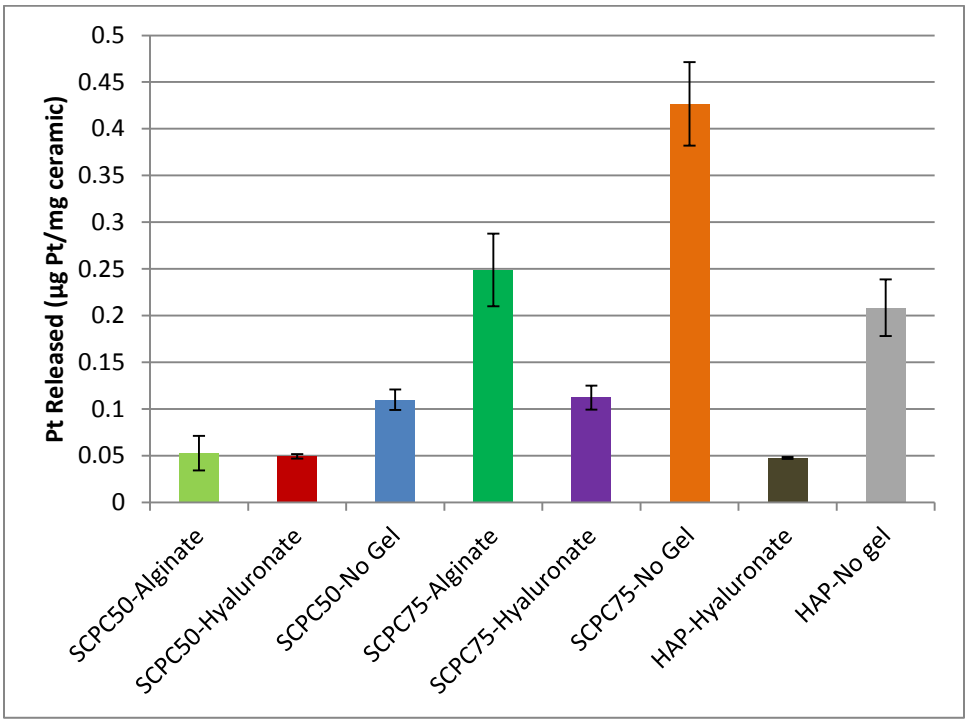

Figure 6: Cisplatin Burst Release at 2 Hours

An anova analysis of the data was performed and the results for the burst release can be seen below in Table 8. A critical value of $P > 0.05$ was used, showing that all of the sample's differences were statistically significant except for the SCPC50 in alginate and SCPC50 in hyaluronate.

Table 8: Statistical analysis of burst release at 2 hours

Materials compared	SCPC50 Alginate & SCPC50 Hyaluronate	SCPC50/ Alginate & SCPC50	SCPC50/ Hyaluronate & SCPC50	SCPC75/ Alginate & SCPC75 Hyaluronate	SCPC75 Alginate & SCPC75	SCPC75 Hyaluronate & SCPC75	Hydroxyapatite Hyaluronate & Hydroxyapatite
P value	0.161723	0.001329	1.69E-05	7.16E-05	0.001007	4.66E-06	3.14E-05
Significant Difference	No	Yes	Yes	Yes	Yes	Yes	Yes

The naked particle cumulative release, as seen in Figure 7, shows the amount of Cis released over time. The data lines up nicely with the drug loading data, showing the SCPC75 with the greatest amount of Cis released while Hydroxyapatite has the least amount. A statistical analysis of the naked particles cumulative release, as seen in Table 9 and Table 10, shows that except for a few time points, the difference in the data is significant. This data also agrees with past studies performed with these materials [].

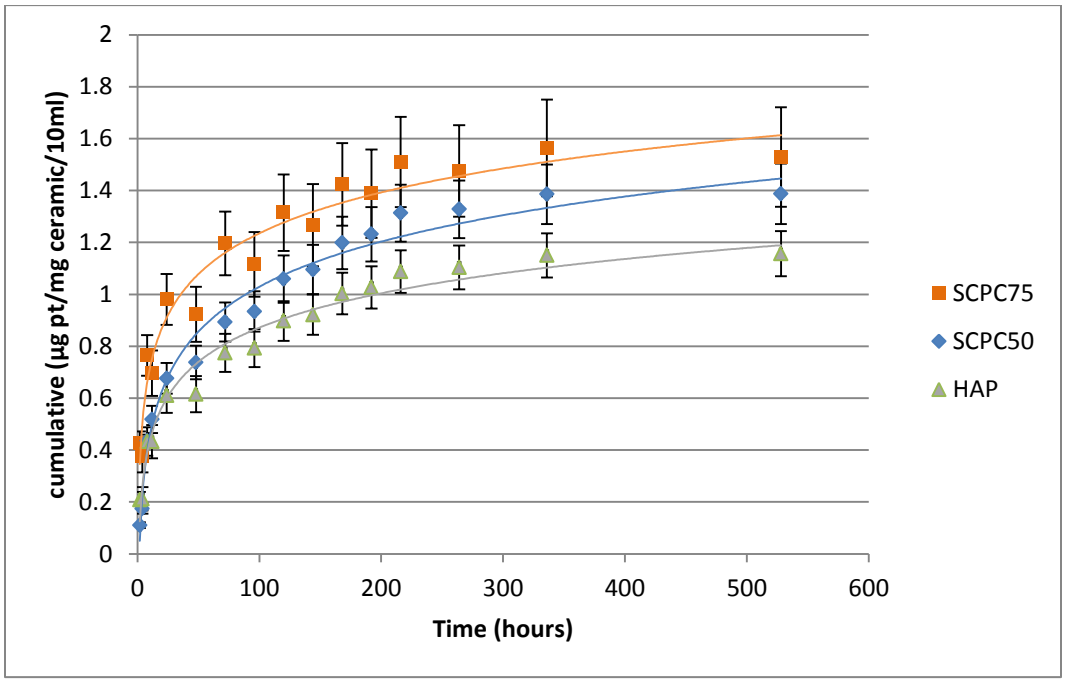

Figure 7: Naked Particle cumulative Pt Release with Logarithmic Fit Line

A Comparison of the cumulative release from SCPC75 can be seen in Figure 8. This figure shows that particles with no gel released a larger amount of Cis than the particles which were combined with gel. The hyaluronate seems to have slowed the release of the Cis to a greater degree than the alginate.

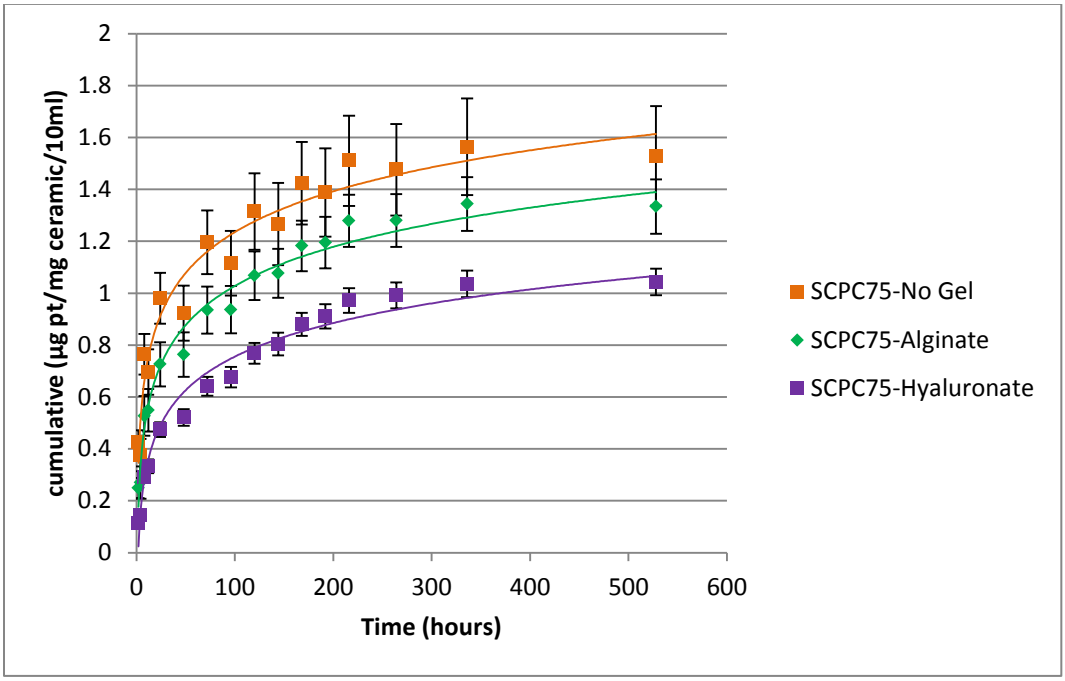

Figure 8: SCPC75 Cumulative Pt Release with Logarithmic Fit Line

The cumulative release data for SCPC50 can be seen in Figure 9. As with SCPC75, the gels seem to have slowed the release of Cis when compared to the naked particles. The difference between the two gels is smaller for the SCPC50 than the SCPC75. While the

overall release is much less for SCPC50 than SCPC75, which aligns with the greater amount of drug loaded on SCPC75.

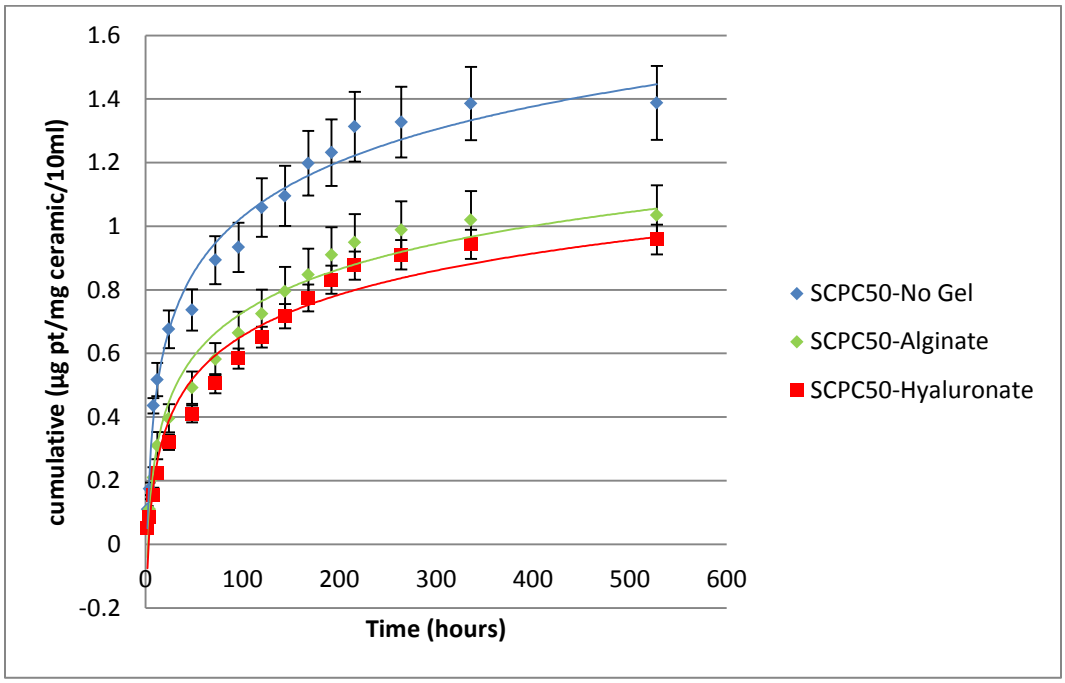

Figure 9: SCPC50 Cumulative Pt Release with Logarithmic Fit Line

The cumulative release values for hydroxyapatite can be seen in Figure 10 below. Again, it shows the slower release of Cis from the gel mixture than from the naked particles. There is no sample using alginate because the calcium in the hydroxyapatite caused the alginate to crosslink and become uninjectable.

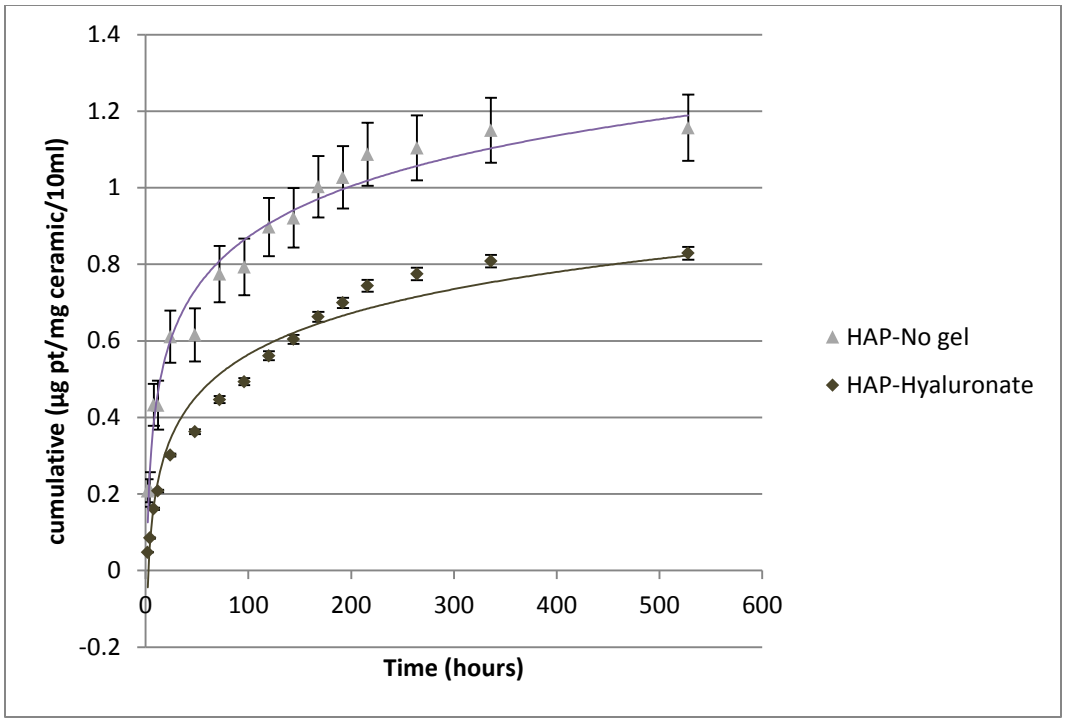

Figure 10: Hydroxyapatite Cumulative Pt Release with Logarithmic Fit Line

Comparing SCPC75 and SCPC50 in alginate, as seen in Figure 11, shows the increased release from SCPC75 and at the same time a similar trend between the two materials.

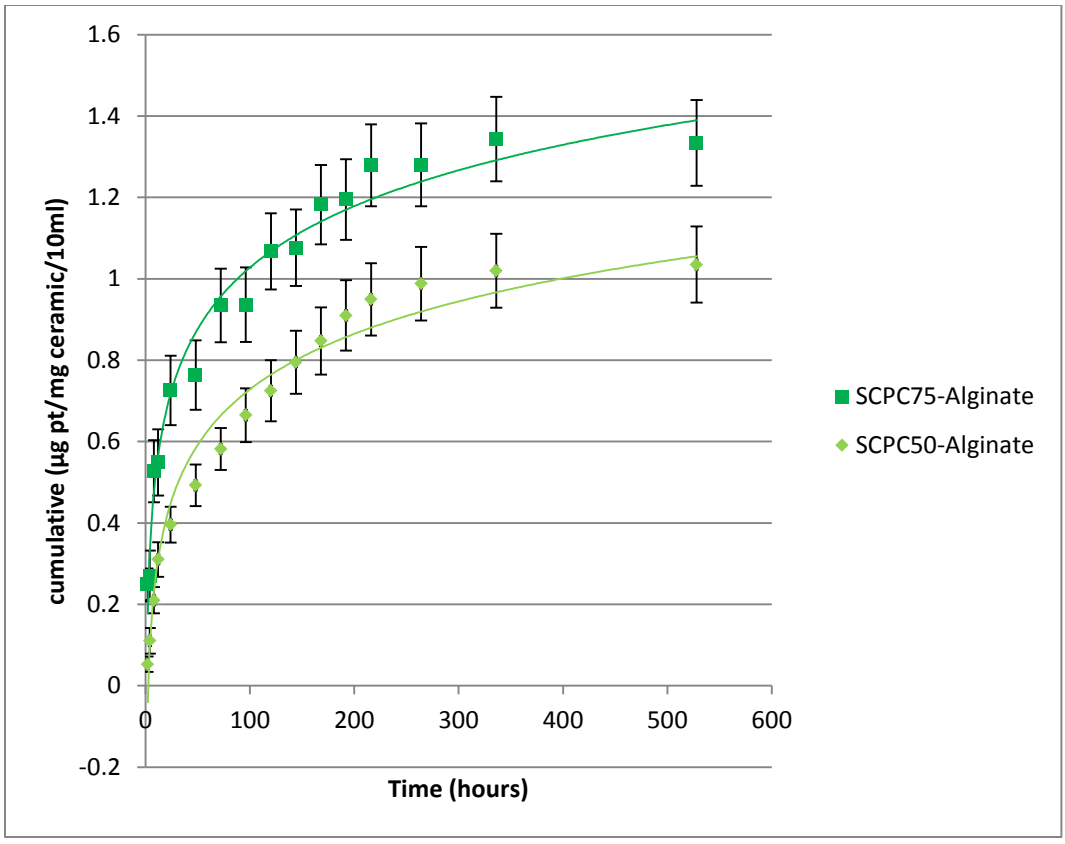

Figure 11: Alginate Cumulative Pt Release with Logarithmic Fit Line

Figure 12 shows the 3 bioceramic samples in hyaluronate. It can be seen that the SCPC75 released the most in these samples, while SCPC50 released the second most and HAP released the least amount of Pt.

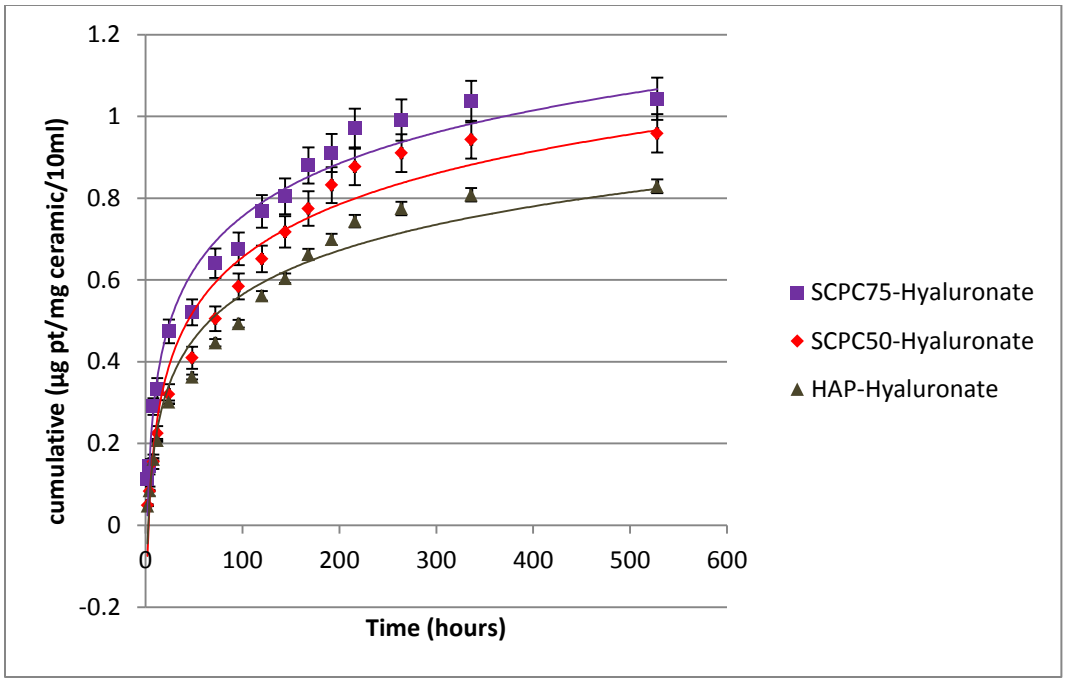

Figure 12: Hyaluronate Cumulative Pt Release with Logarithmic Fit Line

Figure 13 shows the percentage of platinum released from each of the ceramic and gel combinations. It shows that SCPC75 released the highest percentage of the total Pt loaded. When coupled with the fact that SCPC75 also had the most drug loaded, it agrees with the cumulative release charts showing it having the most drug released. It is also interesting to note, that as a percentage released the HAP is greater than the SCPC50, while the total amount of drug released is less due to it being able to carry less.

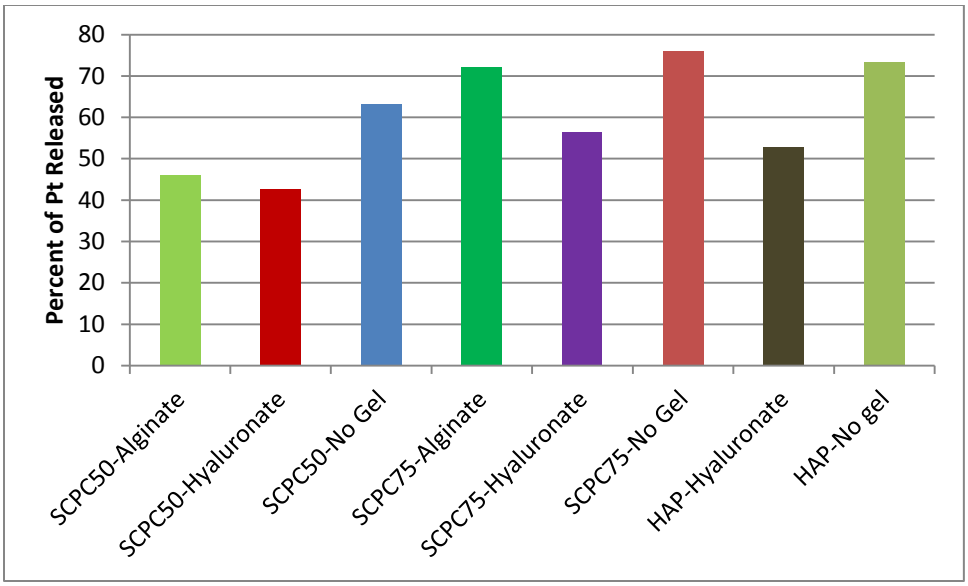

Figure 13: Percent of Platinum Released at 564 hours

Table 9: Anova Analysis P Values for Each Data Point

hours	2	4	8	12	24	48	72	96	120	144	168	192	216	264	336	528
SCPC50A-SCPC50H	0.161723	0.002656	0.000344	1.14E-06	7.56E-06	2.3E-06	0.01454	7.21E-05	0.011934	0.000483	0.010174	0.001614	0.009631	0.002521	0.006757	0.003703
SCPC50A-SCPC50N	0.001329	0.000612	3.36E-06	7.44E-06	1.66E-05	1.68E-06	6.75E-06	4.15E-05	2.98E-05	0.000101	8.95E-05	0.000317	0.000207	7.07E-05	0.000288	0.00048
SCPC50H-SCPC50N	1.69E-05	0.00019	4.02E-08	5.99E-07	2.63E-06	2.64E-07	0.00134	9.75E-07	0.000793	6.51E-06	0.000469	1.13E-05	0.000316	7.07E-05	0.00023	2.11E-05
SCPC75A-SCPC75H	7.16E-05	3.06E-06	4.7E-06	4.57E-06	2.88E-06	1.76E-06	8.28E-07	8.06E-06	4.44E-07	4.35E-06	3.39E-07	2.81E-06	3.84E-07	2.74E-06	4.7E-07	2.47E-06
SCPC75A-SCPC75N	0.001007	0.001997	0.001599	0.007719	0.002141	0.011284	0.003923	0.007288	0.004991	0.009076	0.007176	0.012422	0.010115	0.014684	0.015819	0.017171
SCPC75H-SCPC75N	4.66E-06	7.23E-06	8.97E-06	1.98E-05	2.24E-05	5.55E-05	4.96E-05	0.000112	8.28E-05	0.00018	0.000143	0.000263	0.00021	0.000337	0.000346	0.000407
HAPH-HAPN	3.14E-05	2.47E-06	1.45E-06	8.01E-08	1.22E-06	4.46E-08	2.88E-07	1.03E-08	1.67E-07	8.92E-09	7.55E-08	8.25E-09	5.31E-08	1.2E-08	3.93E-08	1.35E-08
SCPC75A-SCPC50A	7.43E-06	4.22E-07	7.1E-07	1.65E-06	3.16E-07	3.82E-07	8.96E-08	3.34E-06	6.14E-08	2.03E-06	7.61E-08	3.16E-06	1.74E-07	2.9E-06	2.33E-07	2.71E-06
SCPC75H-SCPC50H	5.14E-06	1.98E-05	1.96E-08	1.16E-06	8E-08	5.41E-06	0.001738	0.00013	0.002327	0.000519	0.002699	0.001316	0.003558	0.001804	0.003258	0.001626
SCPC75N-SCPC50N	0.000284	0.001298	0.002348	0.01285	0.00926	0.049453	0.035726	0.055608	0.019555	0.088865	0.037269	0.140264	0.068812	0.162146	0.142958	0.178518
SCPC50H-HAPH	0.211629	0.766466	0.046012	0.002393	8.82E-05	2.6E-05	1.53E-05	1.48E-06	0.102533	0.038421	0.019983	3.23E-06	0.003779	6.47E-06	0.001863	1.54E-05
SCPC50N-HAPN	0.011878	0.085668	0.941694	0.00526	0.039018	0.004885	0.035669	0.002925	0.013079	0.001634	0.003234	0.001109	0.002192	0.001048	0.002065	0.001067
SCPC75H-HAPH	3.74E-06	7.65E-07	1.94E-08	1.93E-07	2.59E-08	1.97E-07	1.63E-07	7.74E-07	3.53E-07	1.14E-06	6.91E-07	1.57E-06	1.18E-06	2.61E-06	1.85E-06	3.65E-06
SCPC75N-HAPN	0.009689	0.000201	0.012883	0.000176	0.000284	0.000276	0.00029	0.000531	0.000381	0.000705	0.000515	0.000913	0.000638	0.001038	0.000985	0.001226

Table 10: Anova Analysis of Data Points, with 1 Being Statistically Significant and 0 not Being Statistically Significant

hours	2	4	8	12	24	48	72	96	120	144	168	192	216	264	336	528
SCPC50A-SCPC50H	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC50A-SCPC50N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC50H-SCPC50N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC75A-SCPC75H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC75A-SCPC75N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC75H-SCPC75N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HAPH-HAPN	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC75A-SCPC50A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC75H-SCPC50H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC75N-SCPC50N	1	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0
SCPC50H-HAPH	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1
SCPC50N-HAPN	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC75H-HAPH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SCPC75N-HAPN	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Chapter 4: Discussion

4.1 Injectability

There were several variables that affected the injectability: gel concentration, needle size, particle concentration, and particle size. The lower concentration of particles was easier to inject. This makes sense since the particle to particle interactions is less and there is more of the lubricating hydrogel available to ease injection. The smaller particles were found to be easier to inject. The larger particles included some that were a large fraction of the inner diameter of the needle, so it is not unreasonable that some of these particles could come together and make injection difficult or impossible. The thinner gels proved easier to inject due to their low viscosity. The larger needles allowed for less obstruction to the flow of the gel-ceramic suspension making for an easier injection. The large needle sizes, 16G, 18G, and 20G were chosen because their inner diameters were larger than the 250 microns of the larger particle size distribution. Moving forward it may be more useful to move to smaller needle sizes that are in use clinically.

It is important to note that only testing for injectability excludes some of the larger picture. What is good for injectability may not be good for the patient it is used on, or for storage. When optimizing for injectability, it is easy to choose a thinner gel, smaller particles, and larger needles. It was observed that while the thinner gels allowed for

easier injection, they also allowed the ceramic to drop out of suspension more rapidly. This would be a problem for storage and distribution of the system. The smaller particles also improve injectability, but they also tend to carry less drug and increase the chances of particle migration out of the injection sight. Larger needles allow for an easier injection, but they can cause more trauma to a patient and create a larger wound track that could cause leakage.

Continuing work is being performed in the lab to further this research. One of the areas targeted for improvement was to determine a way to have a repeatable and observable measurement for injectability. In order to facilitate this, an injectability testing apparatus was designed and fabricated and can be seen in Figure 14. It was designed so a known force could be applied to the syringe plunger and the injection could be viewed through a window in the base. This eliminates some of the qualitative measurements associated with injecting by hand and then grading.

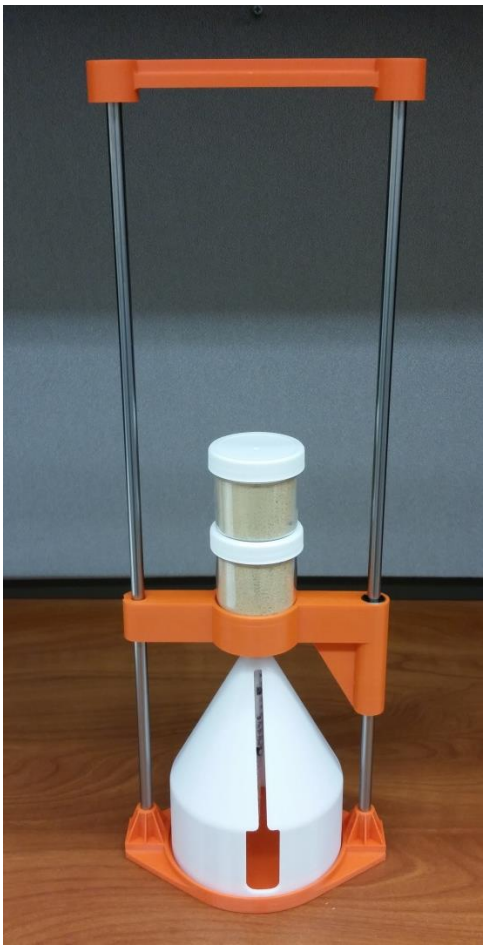

Figure 14: Injectability Testing Apparatus

Work addressing the issue of storage and mixing is also being performed in the lab. Testing to see how long the particles stay suspended in each gel and how that time effects their drug release is needed to determine the viability of storage. Another option is to mix the ceramic and gel immediately before administration. This comes with another set of challenges, as most doctors' offices and hospitals do not have the vortex mixers and other equipment used in the lab at their disposal. So testing into what formulas can be mixed quickly by shaking or simple stirring is an important next step.

4.2 Drug Binding

The results of the drug binding show that SCPC75 contained the most Pt with 2.25μg of Pt per gram of ceramic, SCPC50 contained 1.85μg of Pt per gram of ceramic, and HAP contained 1.57μg of Pt per gram of ceramic. Taking into account the porosity, surface activity, and chemistry of the three materials this is a viable result. SCPC75 contains more pores and a higher surface area than SCPC50, this can be seen in Table 2 [24]. The fact that HAP contained the lowest amount of Pt adsorbed also makes sense; it is a very stable material with a lower porosity.

4.3 Drug Release Kinetics

A feature of drug loaded bioceramics is that there is an initial burst release of drug, followed by a long period of sustained release. Past research has shown that this is due to loosely bonded layers of drug drying on the surface. These layers are easily dissolved into solution. This results in the initial burst release of drug seen in the first hours of Figure 7 through Figure 12. This is useful in the treatment of tumors as it provides a high dose of chemotherapeutic agent early in the administration of the drug delivery system. This would cause a large amount of the cells around the treatment area to die quickly. The sustained release would then continue to release smaller amounts of the drug to continue killing any remaining cells in the vicinity. The sustained release is the result of drug being adsorbed onto the surface of the material and in the pores of the material. These bonds take longer to break and therefore there is a slower smaller release provided.

The samples that were injected with gels showed a much slower release profile when compared to the same ceramic without gel. I suspect that this is due to the gel acting as a buffer and slowing the release of the Pt into the solution and possible slowing the release

from the material itself. It is hard to determine if the release rate from the ceramic into the gel or the buffering action of the gel is the larger cause of the slowed release rate. This is a possible area of study for the future. The alginate gel appears to have inhibited the release of the drug to a lesser degree than the hyaluronate. While both gels had the same concentration of 2.5%, the hyaluronate was much thicker and this may have contributed to the slower release rates from the hyaluronate samples when compared to the alginate samples. The difference in the way the two gels break down in contact with PBS may also have been a contributing factor.

It is important to note that the SCPC75 had not only adsorbed more drug than its compatriots, but at the end of the study, had released a higher percentage of that drug. This is due to its higher porosity and more open pore structure [28]

The use of hydrogels as an aid in injection also opens up the possibility of using it as a drug delivery material as well. Much research is being performed into the use of hydrogels themselves as a drug delivery system [34, 35, 36]. The hydrogels tend to offer a very short term release, ranging from hours to a few days. This could be used in tandem with drug loaded bioceramics to deliver a larger initial dose of drug or to possibly delivery a different type of drug at a different rate. This is an area for additional research in the future.

Drug release kinetics

1. Burst release followed by sustained release why and why
2. 2. Sustained release is dependent on (Dissoultion of the surface, Diffuesion)
3. 3. Comparison of the release kinetics with and without gel

4. Why hyaluronic acid is better than alginate
5. 4. Why SCPC release more : it binds, surface dissolution, Openness of the protsity

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