**Application**

**Residency Grant Project 2016-2017**

**Section I**

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| Project Leader: Ashley Strickland | | | | | Credentials:  MD,  DO, | |
| Male  Female | Date of birth: | | | | NC medical license no.: | |
| Preferred mailing address ( business or  home) | | | City, State, Zip | | | Business Telephone |
| UNC Orthopaedics, CB# 7055, 3140 Bioinformatics Bldg., 130 Mason Farm Rd. | | | Chapel Hill, NC 27599 | | | 919-966-9071 |
| Preferred email | | | Fax | | | Cell Phone |
| Ashley.Strickland@unchealth.unc.edu | | | 919-966-6730 | | | (910)514-6184 |
| Current Residency program | | | | Est. completion date | | |
| University of North Carolina at Chapel Hill | | | | June 24, 2018 | | |
| Program Director | | Director’s phone | | Director’s email | | |
| Robert Esther, M.D. | | 919-966-3340 | | bob\_esther@med.unc.edu | | |
| Program Coordinator | | Coordinator’s phone | | Coordinator’s email | | |
| Karen Gilliam | | 919-966-9071 | | karen\_gilliam@med.unc.edu | | |

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| Additional Project Team Members | | |
| Name | Credentials | Email address |
| Paul Weinhold | MD,  DO, PhD. | weinhold@med.unc.edu |
|  | MD,  DO, |  |
|  | MD,  DO, |  |
|  | MD,  DO, |  |

**Section II**

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| Personal Statement: Please indicate how this grant, if funded, will help toward your career goals and intended area of specialization. Outline your expected career path and how this aligns with the Residency Research Grant program objectives and criteria. (500 words max.) |
| My name is Ashley Strickland, and I am nearing completion of my PGY 3 year of orthopaedic surgery residency at UNC Healthcare. I have recently decided to pursue a fellowship in spine surgery after the completion of my training here. In order to both strengthen my application and further my development as a resident, I am currently designing a research project that I will carry out over the course of the next year. I plan to look at the interaction of bisphosphonate medications and spinal fusion in a rat model. I feel that this will be extremely valuable to me personally, as I will go through the process of developing and carrying out a quality research study while in residency. In addition, it will help to strengthen my surgical skills, as I will be performing surgery on rat subjects. Also, this will help to shed some light on a subject that is poorly understood, as there is very little written in the spine literature on this subject. There have been very few studies looking at these medications in a spinal fusion model. I feel that this study will be an extremely valuable piece of my training, and will hopefully better equip me to understand how to go about carrying out research in the future, if this becomes a part of my practice. After reviewing the aims of this grant program, I feel that my project will fit well with the goals of this grant. I think that the selection committee should also know I have been a resident of North Carolina for my entire life, and have completed all of my training thus far at North Carolina schools. After finishing fellowship, my goal is ultimately to return and practice here in my home state. I feel that North Carolina has given a great deal to me, and I have no intentions of leaving after the completion of my training. I thank you for your time and consideration, and I look forward to hearing from you all, should you decide to assist in funding my research endeavor. |

**Section III – Details of the proposal**

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| Abstract summary |
| The current literature is inconclusive as to the effect of systemic administration of bisphosphonates on spinal fusion. In vitro evidence that bisphosphonate can increase osteoblast proliferation and maturation and in vivo studies showing improved osseointegration with local delivery of bisphosphonates, suggest that local delivery of bisphosphonates to the spinal fusion site may be an effective method to enhance fusion outcome while minimizing any systemic side effects. The purpose of this study is to evaluate the influence of zoledronic acid locally applied to bone graft on spinal fusion outcome. An established posterolateral fusion model in the rat will be used with the animals followed for 6 weeks. Animals will be divided into 3 groups with the bone graft placed at the spinal fusion site soaked as follows: 1) 0.1ml saline, 2) 0.1ml of zoledronic acid (0.025mg/ml in saline), 3) 0.1ml of zoledronic acid (0.25mg/ml in saline). After euthanasia, spinal fusion will be evaluated by radiographs, by bone volume fraction at the surgical site from micro-CT scans, by 4 point bending load to failure of the spinal segment and by qualitative histological evaluation. It is anticipated both zoledronic acid groups will improve the incidence of radiographic union, increase bone volume fraction and improve the bending failure load with respect to the saline control group. |
| Outline of the problem |
| Spinal fusion is one of the most commonly performed orthopaedic procedures, representing more than 400,000 procedures annually in the United States. The success of this procedure depends on the formation of a solid fusion mass, which relies heavily on the interaction of the osteoblast-osteoclast complex. The most frequently encountered complication of spinal fusion is pseudoarthrosis occurring in 10 to 30% of cases. This leads to several problems, including chronic pain and need for re-operation.  The most common metabolic disease of bone is osteoporosis, which affects an estimated 10 million patients in the United States. One of the most common medication classes utilized to manage this condition is the group of medications known as bisphosphonates. These are anti-resorptive medications that have a 3-fold mechanism of action. They work to inhibit the process of osteoclastogenesis in the bone marrow, they decrease the activity of osteoclasts on the bone surface, and also decrease the lifespan of osteoclasts by increasing the apoptosis rate of this cell line. The targets of action of these medications have motivated many to ask what effect they may have on the biology of spine fusion. Thus far, there has been very little investigation in this area. It should be an area of focus for future research for two reasons. One, these medications are becoming increasingly utilized in the general population, and many of these patients will undergo spinal surgery at some point. For this reason, we should better understand the effect that these medications could potentially have in this population, and whether it is safe, or potentially advantageous to continue these medications in patients undergoing spinal fusion. Also, there is growing data to suggest that the administration of these medications may be beneficial in the presence of spinal fusion, which may lead to an indication to utilize these medications to improve spinal fusion rates in the future.  Bisphosphonates are being studied in the presence of acute fracture healing in animal models. In these situations, they have been shown to increase the volume of fracture callus formed, but with less mature bone formation. The effect on the eventual strength of the healed fracture is not yet fully understood, as several studies have had conflicting results in this regard. |
| State of the art in this field |
| There are very few studies that look at spinal fusion and bisphosphonate medications. The only human study was a prospective trial that looked at alendronate use in patients undergoing a single level posterior lumbar interbody fusion, and they showed a significant increase in fusion rate in patients on alendronate, with a fusion rate of 95% as opposed to 65% in the control group. There was also significantly less cage subsidence, and significantly fewer vertebral compression fractures at adjacent levels(1). There is one study that has looked at spinal fusion in a rat model, which has shown promising results with respect to the rate of spinal fusion in rats that have been treated with injected zoledronic acid. They showed that a trend toward increased spinal fusion rates with increasing dose, with the high dose reaching statistical significance with a small sample size. They were also able to show increased fusion mass volume and density in all of their experimental groups (2).  There have been several recent studies in the total joint literature that have investigated the rate of allograft resorption as well as new bone formation in the presence of zoledronic acid and other bisphosphonates. They have shown that at varying concentrations, bisphosponates were able to both decrease resorption of allograft, and increase the amount of new bone formation surrounding a titanium implant. This data could be utilized to develop a hypothesis that use of local bisphosphonates at the graft site of a posterolateral spinal fusion, would result in decreased graft resorption rates, and potentially the formation of a more solid fusion mass as a result.   1. Nagahama K, Kanayama M, Togawa D, Hashimoto T, Minami A. Does alendronate disturb the healing process of posterior lumbar interbody fusion? A prospective randomized trial. *Journal of Neurosurgery: Spine*. 2011 14: 500-507. 2. Yasen M, Li X, Jiang L, Yuan W, Dong WCJ. Effect of Zoledronic Acid on Spinal Fusion Outcomes in an Ovariectomized Rat Model of Osteoporosis. *Journal of Orthopedic Research*. September 2015 |
| Past research of the applicant in this field |
| UNC has currently utilizing local administration of local bisphosphonates in a rat model looking at osteointegration of porous ingrowth titanium implants which has shown promising results with respect of osteointegration of these implants in an ongoing biomechanical study. There has been no previous work in the realm of spine surgery and bisphosphoneates at UNC. |
| Open questions |
| Do bisphosphonates have an effect the rate of spinal fusion?  Do bisphosphonates alter the resporption rate of local autograft in a spinal fusion model?  Do bisphosphonates have an effect on the ultimate strength of a posterolateral spinal fusion?  Does local administration of zoledronic acid affect the microscopic structure of a spinal fusion mass? |
| Hypothesis |
| Locally administered bisphosphonates will decrease osteoclast resorption of iliac crest autograft at the site of posterolateral spinal fusion in a rat model. Biomechanically, the resulting improved fusion mass will result in a stronger fusion. Radiographically, bisphosphonates will result in higher incidence of radiographic fusion on AP plain films, and will result in larger fusion mass volume and density on micro-CT analysis. |
| What are the aims you want to reach with this study? |
| The aim of this study is to determine the safety efficacy of local administration of zoledronic acid in a rat model of posterolateral spinal fusion. We anticipate that we will be able to show that local administration of zoledronic acid will both decrease the rate of resorption of local autograft as well as increase the rate of successful spinal fusion. |
| Anticipated results |
| It is anticipated both zoledronic acid groups will improve the incidence of radiographic union, increase bone volume fraction and improve the bending failure load with respect to the saline control group. |
| Study subjects, specimen or materials |
| The subjects in this study will be 36 Sprague Dawley rats, twelve each in a control and two experimental groups. Other materials include consumable surgical instruments and medications (Buprenorphine and acetaminophen and zoledronic acid). |
| Effect and outcome variables |
| The primary outcome of this study will be 4 point bending strength of the spinal fusion segment after the subjects have been euthanized at 6 weeks post-op. Secondary outcomes that will be analyzed include fusion mass volume and fusion mass density, which will both be calculated utilizing micro-CT analysis. In addition, 3 rats from each group will be sent for microscopic analysis as a qualitative to better characterize any difference in fusion mass structure that may be observed as a treatment response. |
| Methods for taking measurements |
| All of subjects will first be sent to the UNC Biomedical Research Imaging Center for micro-CT analysis with 3 dimensional reconstructions. The imaging results can then be utilized qualitatively to assess for successful fusion. In addition the scanner data can be used to calculate both the volume and density of the fusion mass in each subject. After imaging, 3 subjects form each group will be randomly selected and sent for qualitative microscopic analysis. Microscopic analysis will not be utilized as a primary outcome measure in this study. The remaining 9 subjects in each group will be analyzed in the UNC Orthopaedics Biomechanics Lab for four point bending strength analysis as described by Metzger, et al. This will be our primary outcome measure in this study. |
| Methods for data management and analysis (including biostatistical check) |
| This portion of the project is still under development, update can be provided once this information is available if this project is awarded funding. |
| Estimation of sample size and power |
| Sample size calculated by power analysis based on prior biomechanical study of posterolateral spinal fusion 4 point bending analysis results (1). The power analysis was carried out with an alpha of 0.05 and a power of 0.8 with an anticipated 30% difference. This resulted in a sample size of 8 per group. We will plan to include 12 rats in each group, 9 for biomechanical analysis and an additional 3 that will be sacrificed for histology. This will also allow for the loss of one rat in each group, while allowing us to maintain a sufficient sample size.  1. Metzger MF, et al. The Relationship Between Serum Vitamin D Levels and Spinal fusion Success. *Spine* 2015 40(8) 458-468. |
| Animal model  If an in vivo animal model is used in the planned research work, please describe the model in detail. The description should include: anesthesia protocols, treatment protocols, pain management, surgical techniques, post-operative care, criteria for removal from the study if necessary, and euthanasia protocols.  AAALAC accreditation (Association for assessment and accreditation of Laboratory Animal Care International)  www.aaalac.org  Please indicate whether the institution (main applicant and co-applicants) is AAALAC accredited and specify in which institution the animal research will be carried out. If the institution is not AAALAC accredited, please detail what agency and standards are used to oversee animal use and care. |
| The surgeries and animal care will take place at UNC in the Dental Research Facility.  AAALAC International Approval #329  Procedures:  Instruments will be sterilized using steam sterilization. On the day of surgery each rat will be anesthetized using inhalation isoflurane by vaporizer and weighed. The anesthesia will be monitored with respiratory rate and toe pinch reflex. The rats will be placed over a heating pad for body temperature control. Clippers will be used at a location separate from the sterile field to remove excess hair at the surgical site on the lumbar region and the base of the tail. The rat will be placed over a heating pad for body temperature control. The surgeon will wear a clean lab coat, shoe covers, surgical bonnet, sterile gloves, and surgical mask. The wrists of the sterile gloves will be pulled over the sleeves of the lab coat using aseptic technique so the sleeves of the lab coat will not interfere with the sterile field. The surgeon will sanitize his hands with a surgical scrub for three minutes prior to gloving with sterile gloves. The surgical site will be disinfected with three alternating swabs each of betadine and isopropyl alcohol. Scrubbing will be performed starting at the center of the surgical site and moving in concentric circles toward the edges. The scrubbed surgical area will be draped with a sterile disposable drape. Each rat will be initially anesthetized followed by a single dose of a prophylactic antibiotic. Specifically, we will use a single dose of ceftriaxone injected subcutaneously. This should provide 24-hour coverage. First, the tail will be removed from the animal, and cancellous autograft will be harvested from the tail vertebrae to be used as the graft source for the posterolateral fusion. The posterolateral spinal fusion will then be carried out. Each rat will undergo a bilateral Wiltse muscle splitting approach to the transverse processes of L4 and L5 through a single dorsal skin incision. The harvested tail vertebra autograft will be then be treated with either a saline control or a pre-determined experimental dose of zoledronic acid [A) 0.1ml saline, B) 0.1ml of zoledronic acid (0.025mg/ml in saline), C) 0.1ml of zoledronic acid (0.25mg/ml in saline)] and a standardized volume of graft will be placed at each fusion site following decortication of the TP’s using a high speed burr. The incision will then be closed using deep vicryl suture and wound clips.  Each rat will receive its buprenorphine SR for postop analgesia either intraoperatively or during recovery. Rats will be given 5mls of warmed Ringer's lactate solution by SQ injection. The animals will be monitored until they recover from anesthesia. The rats will then be returned to their cages. All rats will receive adequate analgesia during the postoperative period and will be monitored closely for signs or symptoms of infection and/or poorly controlled pain. The rats will be observed after surgery until they are awake and able to ambulate. The animals' body temperature will be maintained with a heating pad during the recovery period. Post-operative analgesics will be given in the form of acetaminophen (200 mg/kg) as 5 mL of elixir mixed with 100 mL of the animal's drinking water for 7 days post-operatively. Acetaminophen will be present in the water of all animals for three days prior to each surgery so that they are able to adjust to the taste of the elixir. Furthermore, animals will receive a subcutaneous injection of buprenorphine SR to provide analgesia for three days post-operatively. Because a single dose of buprenorphine SR can induce pica in rats, digestible paper bedding will be used instead of the standard bedding for 1 week post-op to reduce the change of gastric obstruction. Additionally, a smaller dose of buprenorphine (0.5-0.8mg/kg) will be used to minimize the risk pica based on our past experience. The rats will also receive a one-time injection of ceftriaxone (SQ injection, 25mg/kg daily) immediately after surgery for infection prophylaxis. Post-operative care will consist of daily observation 3-5 days post-op and then weekly until the animals are euthanized, specifically to check on the well being of the animal. Animals exhibiting signs of excess infection or distress will be immediately removed from the study. At 7 to 14 days, wound clips will be removed. The rats will then be housed for a period of 6 weeks. At 6 weeks post-op, the rats will be euthanized for analysis.  The rats will be euthanized using carbon dioxide overdose, followed by a thoracotomy 6 weeks after the initial surgical procedure. Rats appearing to have severe infections/pain will be immediately euthanized. Our criteria for euthanasia consist of wound dehiscence, malaise or activity depression as evidenced by reluctance to move, excessive wound discharge, significant lethargy, or weight loss of more than 15 percent body weight. |
| Relevance of the project |
| This study will potentially have several important clinical implications in the future. This study takes an idea from the total joints basic science literature, that local administration of bisphosphonates decreases local bone graft resorption, and applies this to a spinal fusion model. To our knowledge, this will be the first study to look at local administration of zoledronic acid to cancellous autograft in a spinal fusion model. This study will contribute to the already growing literature indicating that bisphosphonate therapy may not only be safe in the spinal fusion patient, but also may decrease resorption of graft material, contributing to an increased overall fusion rate. |
| Time schedule |
| 6/16 to 8/16: Funding and IACUC approval for surgical plan.  9/16 to 11/16: Surgical technique training, and rehearsal of surgery on cadaveric specimens.  2/6/17 to 3/27/17: Experiment will be carried out.  3/27/16 to 4/8/17: Statistical analysis. |
| Relevant literature by the investigators |
| No published research in this area of research. |
| Relevant literature by other authors |
| 1. Nagahama K, Kanayama M, Togawa D, Hashimoto T, Minami A. Does alendronate disturb the healing process of posterior lumbar interbody fusion? A prospective randomized trial. *Journal of Neurosurgery: Spine*. 2011 14: 500-507. 2. Yasen M, Li X, Jiang L, Yuan W, Dong WCJ. Effect of Zoledronic Acid on Spinal Fusion Outcomes in an Ovariectomized Rat Model of Osteoporosis. *Journal of Orthopedic Research*. September 2015 3. Metzger MF, et al. The Relationship Between Serum Vitamin D Levels and Spinal fusion Success. *Spine* 2015 40(8) 458-468. 4. Hirsh BP, Unnanuntana A, Cunningham ME, Lane JM. The effect of therapies for osteoporosis on spine fusion: A systematic review *The Spine Journal* 2013 13:190-199. 5. Jakobson T, et.al. The Effect of Soaking allograft in Bisphosphonate. *Clin Orthop Relat Res*. 2010 468:867-874. 6. Bobyn JD, et.al. Local Alenronic Acid Elution Increases Net Periimplant Bone Formation: A Micro-CT Analysis. *Clin Orthop Relat Res*. 2014 472:687-694. 7. Gezici AR, et.al. The Effect of Risedronate on Posterior Lateral Spinal Fusion in a Rat Model. *Journal of Korean Neurosurgical Soc.* 2009 46: 45-51. 8. Rajaee SS, HW Baem LE Kanim, RB Delamarter. Spinal fusion in the United States: analysis of trends from 1998 to 2008. *Spine* 2012 37(1): 67-76 |

**Section IV – Budget for proposed project period**

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| --- | --- | --- | --- |
| **Personnel** | | | **Amount** |
| Surname / First name | Academic qualification | Effort in % |  |
| Strickland/ Ashley | MD | 1 | 0(cost shared) |
| Weinhold/Paul | PhD | 1 | 0(cost shared) |
| **Total cost for personnel** |  |  | **0** |

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| --- | --- |
| **Material** | **Amount** |
| Devices, equipment, extension to existing equipment, etc. | **NA** |
|  |  |
|  |  |
|  |  |
| **Total cost for material** |  |

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| --- | --- |
| **Supplies** | **Amount** |
| Itemize below |  |
| Animal purchase and boarding | $2580 |
| Histology | $225 |
| Radiograph | $150 |
| Buprenorphine and acetaminophen and zoledronic acid | $355 |
| Surgical supplies | $360 |
| **Total cost for supplies** | **$3670** |

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| --- | --- |
| **Rental/Use of equipment** | **Amount** |
| Itemize below |  |
| Micro-CT Scanning | $1330 |
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| **Total cost for rental equipment** | **$1330** |

**Section V**

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| If selected for participation in the program, the grantee agrees to conduct herself/himself professionally according to the principles of medical ethics and to be governed by the Bylaws of the North Carolina Spine Society. | | | | |
| Applicant’s signature: |  | | Date: |  |
| Program Director’s signature: | |  | Date: |  |

To be considered for the 2016-2017 grant year,   
**this application and the applicant’s CV are due by 5:00 pm on June 13, 2016.**

**Please sign your completed form and return it along with your CV by email, mail or fax to:**

NCSS, PO Box 27167, Raleigh, NC 27611 | Fax: 919-833-2023 | [ncspine@ncmedsoc.org](mailto:ncspine@ncmedsoc.org)