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Purpose: To evaluate bone graft substitutes used in spine fusion surgery and determine the feasibility of studying the use of adult stem cells.

Hypothesis: Using a well designed, randomized clinical trial to compare bone graft substitutes used in spine fusion surgery will help determine the best alternative to autologous bone graft.

Design: Retrospective data on two bone graft substitutes will be evaluated. A protocol for studying stem cells in spine fusion will be drafted and the feasibility of implementing the trial will be analyzed.

Results: It is difficult to design a randomized clinical trial to investigate a new surgical technique. The lack of standardization among spine surgeons makes it difficult to control for confounding variables.

BONE MARROW ASPIRATE VS. BONE MORPHOGENETIC PROTEIN (RHBMP-2) IN
MULTILEVEL ADULT SPINAL DEFORMITY SURGERY AND THE FEASIBILITY OF
USING ADULT MESENCHYMAL STEM CELLS

INTERNSHIP PRACTICUM REPORT

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CHAPTER I

INTRODUCTION

I conducted my internship at the Baylor Scoliosis Center located on the campus of Baylor Regional Medical Center at Plano, where I worked as a clinical research coordinator. The Baylor Scoliosis Center's clinical practice opened in 2005 as the first center in the Dallas/Fort Worth metroplex devoted to the treatment, surgery, and care of advance spine curvature in adults. As a national referral center, the providers at the Baylor Scoliosis Center performed more than 400 surgeries to straighten the spine of scoliosis patients in the last year. In addition to performing surgical correction, the team at the Scoliosis Center offers its patients continuity of care with a long-term treatment plan.

In 2006, through the Baylor Research Institute, scoliosis research studies commenced at the Baylor Scoliosis Center. With the commitment to advanced research efforts, the research team consisting of two clinical research coordinators and one clinical research assistant, manages nine active studies. Research in the areas of pain, spine fusion, genetics, possible scoliosis biomarkers, surgical techniques, instrumentation, and surgical outcomes are being investigated. The main goal is to improve the treatment and quality of life of all spine deformity patients. Currently all of the ongoing studies are observational or review studies. During my internship experience I had the opportunity to participate, observe, and assist in various aspects related to the management of clinical trials. At the start of my internship, I reviewed some of the available

literature on scoliosis, its treatments, outcomes, and complications. I also reviewed the Scoliosis Research Center study protocols and became familiar with the various clinical outcomes, questionnaires, and databases used for research purposes. I observed the research study process including patient consent, data collection, and follow-up. In addition, I assisted with the preparation of various IRB documents and various other case report forms.

As part of my internship, I was asked to assist in the drafting of the manuscript for one of the studies that had just completed enrollment. The manuscript was based on a retrospective comparison analysis of Bone Marrow Aspirate (BMA) in combination with a carrier sponge (HEALOS®) versus Bone Morphogenetic Protein-2 in surgically treated scoliosis patients. The hypothesis of this trial was that the two grafting alternatives were equivalent in fusion success and clinical outcomes.

Based on extensive literature review and data from the HEALOS® study, it became apparent that scoliosis research was in need of more well designed, randomized clinical trials. Therefore, this study looked at the feasibility of implementing an investigational, randomized clinical trial using adult Mesenchymal stem cells versus bone marrow aspirate in surgical arthrodesis. I designed an investigational study protocol and presented it to the Baylor Institutional Review Board (IRB) for approval. Outcomes measures for this study were complications with design of the study and feedback from the IRB.

CHAPTER II

INTERNSHIP SUBJECT

Background and Literature Review

Scoliosis

Scoliosis is a complex three-dimensional disease of the spine. The scoliotic spine has an abnormal side to side curvature and is also generally associated with a rotational deformity. It is further radiographically defined as a curve greater than or equal to ten degrees measured by the Cobb method with rotation. Scoliosis can be found at birth, due to genetic causes, developed during childhood, or developed late in life due to degenerative disc or joint disease (8). The prevalence of scoliosis was estimated to range from 1.4 to 9 %. Girls are more likely to have scoliosis. The incidence is greater in the children of women with scoliosis and particularly in the daughters of these women (16).

Radiographic imaging is the main diagnostic tool. In particular, standard anterior/posterior (AP) and lateral full-length spine x-ray films are used to measure the Cobb angle (38). In addition, in patients with scoliosis, the shoulders or pelvis may not be level, or waist asymmetry may be noted. Scoliosis may be suspected when one shoulder appears higher

than the other or pelvic asymmetry is noted. In some cases, as the spine curves abnormally, the involved vertebrae are forced to rotate. If rotation occurs at the thoracic level of the spine, vertebral turning impacts the rib cage and may result in rib and scapular prominence. The rib hump, or prominent lumbar curve, can be accentuated by having the patient lean forward from the waist, permitting the arms to hang down; the examiner then views the spine. The rib hump can be quantified by using a scoliometer, which permits measurement of angular deformity.

The symptoms and physiology in adult patients with spinal deformity are more complex than for childhood spinal deformity (9). Adult scoliosis can either be carried over from childhood or develop de novo as a result of degenerative changes in the spine leading to deformity (16). Childhood scoliosis that progresses into adulthood may lead to many significant problems. Untreated scoliosis in the adult can lead to painful spinal osteoarthritis, progressive deformity, spinal stenosis with radiculopathy, muscle fatigue, and psychological effects of living with a visible deformity (16). This can be especially disabling in the older population and can impact daily activities.

Various methods have been used to treat scoliosis, including observation, bracing, and operative treatments. Observation is usually reserved for patients who have curves less than 25 degrees. Skeletally immature patients with curves less than 25 degrees should be examined every 6 to 12 months. Skeletally immature patients with curves greater than 25 degrees generally require bracing, which is used to stop curve progression rather than correct curves. Curves greater than 40 degrees are difficult to control with bracing and are at greater risk for progression. One study found that 68% of patients had progression of their curvature after skeletal maturity for curves greater than 50 degrees while curves less than 30 degrees tended not to progress (16). In growing children, operative treatment is indicated for severe deformity

(curves greater than 50 degrees) that progressed despite bracing and uncontrolled pain. In adolescents the decision for surgery is highly driven by the degree of deformity. Operative considerations for spinal deformity in adults include: pain, functional limitation, neural element compression, degree and location of deformity, curve magnitude and deformity progression (16). While the incidence of complications in adolescent patients is quite low, the risk of major complications in adult scoliosis surgery is reported to be upward of 30% with increased rates found in association with more complex cases, older patients, and patients with coexisting medical conditions (37, 59).

Spinal Fusion

Spinal fusion is a surgical method of treating severe scoliosis patients. The procedure stabilizes the spine by fusing adjacent vertebrae together, eliminating normal intervertebral movement, and forming a solid mass of bone incorporating the adjacent vertebrae. The procedure alleviates the back pain caused by spine curvature and prevents further progression of deformity. The goals of surgery for spinal deformity are to correct or to improve deformity, minimize morbidity or pain, maximize postoperative clinical outcomes, prevent progression of the curve, and improve the function of the lumbar spine. Surgical approach may include anterior, posterior, or combined anterior and posterior fusion depending on a variety of factors (28, 32, 35).

The basics of spinal fusion involve the use of some type of fixation hardware to correct the deformity and to hold the spinal bones together while placing graft material between vertebral bodies in the anterior column or in the posterior interlaminar region to act as a bridge and scaffold for osteoblasts and to promote ingrowth of new bone. The hardware keeps the spine

from moving while new bone forms to bridge the vertebrae. Most spinal instrumentation constructs have two elements. The first element is a device that solidly attaches to the vertebral bodies, such as screws placed in the pedicles or vertebral bodies. The second element is a device that traverses the vertebral segments to be fused and locks to the screws. Screws are placed at multiple selected sites along the deformity, and distraction or compression is applied to correct the curve. Once biologic fusion occurs, the bones are permanently locked together and the hardware is no longer necessary. However, it is generally left in place to avoid another procedure to remove it (10, 90).

The long-term success of any operative procedure for scoliosis depends on a solid arthrodesis, or fusion. The success of spinal fusion depends on surgical preparation of the fusion site, systemic and local factors, ability of the graft material to stimulate a healing process, and biomechanical features of the graft positioning. The surface of the bone and the facets should be decorticated to provide a large, maximally exposed surface area for vascular ingrowth and to allow delivery and attachment of osteoprogenitor cells (48, 57). Fusion rates can range from 46% to almost 98% (11). These rates may increase with improvements in surgical techniques, bone grafts, and hardware.

It is not always obvious when a solid fusion has been obtained as opposed to a nonunion or pseudarthrosis (12). Accomplishment of fusion is defined radiographically by bridging of trabecular bone connecting the two vertebral bodies, no angular motion in excess of five degrees, no sagittal translation in excess of three mm, and no radiolucencies that involve more than half of the interface between the vertebral end plates (55, 56). Radiographs are accurate in assessing success or failure to attain solid fusion only 70% of the time (60). Although surgical exploration

remains the “gold standard” for definitive fusion success, CT scans are also an acceptable method (8).

Pseudarthrosis (PSAR)

Despite modern advanced techniques, achieving multilevel spinal fusion in adult deformity patients continues to pose a significant challenge. The failure of arthrodesis, or pseudarthrosis (PSAR), is defined as the documented failure of solid fusion 1 year after operation (14). The rate of nonunion, or pseudarthrosis, for spinal fusion has been reported to be between 5% and 35% (14, 27). In adult patients, the pseudarthrosis rate is substantial especially if a long fusion is being attempted in patients older than the age of 55 years (27). Kim *et al* reported the overall prevalence of nonunion following long adult spinal deformity fusion with instrumentation was 24% (55, 56). Failure to achieve solid fusion may lead to pain, loss of correction, progression of deformity, instability, and potential neurologic injury (57, 84). In addition, a nonunion frequently leads to unsatisfactory resolution of clinical symptoms and usually results in greater medical costs due to the need for additional surgeries and increased morbidity (13).

Factors contributing to pseudarthrosis include inadequate surgical technique, failure to neutralize excessive motion, or shear stresses at the segments to be fused (84). Implanting less of the normal volume of graft, absence of decortications in the host bed, and using inadequate instrumentation may contribute to disappointing fusion rates (79). In addition, host-specific factors such as age, gender, comorbidities, and smoking have been shown to affect the rate of fusion. Length of fusion construct and poor local bone quality has also had negative impact on surgical outcomes (57).

Diagnosis of PSAR is based on clinical presentation and imaging studies. High suspicion for PSAR may necessitate re-exploration and fusion (79). In general, criteria used to detect pseudarthrosis are: (1) loss of fixation, such as implant breakage, dislodgement of rods or hooks, or halo around pedicle screws; (2) progression of deformity with or without pain; (3) subsequent disc space collapse; (4) motion during surgical exploration (56). In addition, patients with PSAR score significantly lower on quality of life index questionnaires (SRS and ODI). Overall, a high degree of clinical suspicion along with CT scans may be the most reliable method for PSAR diagnosis (79).

The management of pseudarthrosis in the adult scoliosis patient depends upon the level of the nonunion, the presence or absence of deformity, and the presence or absence of pain. A painful pseudarthrosis associated with progressive deformity and loss of fixation is a clear indication for spinal revision surgery (16). Treatment of patients with symptomatic pseudarthrosis involves a second attempt at fusion and may require an approach different from that of the index surgery as well as the use of additional instrumentation, bone graft, and osteobiologic agents (79). Although asymptomatic pseudarthrosis are well documented, Steinmann and Herkowitz found pseudarthrosis to be the contributing factor in 78% of those patients requiring reoperation (84).

Bone Graft

The majority of traumatized tissues heal with a fibrous scar. The cells and structure of the scar are not normal and are unable to fully assume the function of the tissue. In contrast, bone is capable of true cellular, morphologic, and functional regeneration (27). Thus, bone is a unique tissue because it is able to form normal bone following fracture and the disruption of its matrix.

Bone regeneration is required to achieve spinal fusion. The initial phase of bone healing is characterized by an inflammatory response with the release of cytokines and various growth factors (58). These proteins facilitate the proliferation of marrow stromal cells adjacent to the wound site as well as recruitment of undifferentiated mesenchymal stem cells (MSCs) from nearby tissues. MSCs then proliferate and differentiate into osteoblasts (46, 52, 58). Mature osteoblasts secrete the type I collagen and non-collagenous proteins of the bone matrix and regulate the mineralization process of bone formation (82).

Thus, the biological processes involved in bone regeneration require three critical elements as follows: an osteogenic potential that is capable of directly providing cells to the newly forming bone, osteoinductive factors that are able to cause osteoblastic differentiation of osteoprogenitor stem cells, and osteoconductive scaffold that facilitates neovascularization and supports the ingrowth of bone (66). For fusion to succeed, osteoprogenitor cells must differentiate into osteoblasts, populate the fusion matrix, survive in the fusion environment, and deposit bone.

The choice of graft material will influence the outcome of a spinal fusion. Osteoconductive materials serve as nonviable, passive scaffolding to promote vascular invasion and a surface onto which osteogenic cells can attach for new bone formation (58). The three-dimensional scaffolds play a critical role in both cell targeting and cell transplantation strategies. Scaffold matrices serve as space holders; provide surfaces that facilitate the attachment, survival, migration, proliferation, and differentiation of stem cells and progenitors; and provide a void volume in which vascularization, new tissue formation, and bone remodeling can occur (71, 73). In addition, an osteoconductive matrix provides a vehicle for the delivery of cells and proteins into the graft site (75).

Osteoinductive materials are ones that provide biologic signals necessary to mediate recruitment and differentiation of cell types essential to bone formation (39). Osteoinductive substances also induce differentiation of local mesenchymal stem cells (MSCs) into mature osteoblasts and promote neovascularization. Osteogenic materials are those that contain viable osteoprogenitor and osteoblast cells that can lay down new bone matrix. These bone-forming cells participate in the early stages of the healing process to unite the graft to the host bone (14). While osteoprogenitor cells and MSCs are capable of direct osteosynthesis, no osteoconductive material or osteoinductive stimulus will be effective in the absence of osteogenic cells (82). Osteogenic cells may be added to the surgical site or they may migrate into the graft site from surrounding tissues (70, 71). The ideal bone graft material possesses all the three critical elements (osteoconduction, osteoinduction, and osteogenic potential). In patients undergoing spine fusion, the choice of bone-graft substitutes remains an emotional rather than scientifically driven decision for many.

Autograft

Autogenous bone is considered the most successful bone graft material and is considered the “gold standard” of graft materials. An autograft is bone taken from one site, most commonly the iliac crest, and transported to another site within the same individual. Advantages of autograft include immunogenetic compatibility, absence of disease transmission, and biomechanical strength (82). Inherent to this material are all three of the ideal transplant properties: osteogenic potential, osteoinductivity, and osteoconductive.

However, high rates of postoperative pain and morbidity often result from the grafting procedure as well as limited fusion rates due to mechanical and biologic deficiencies of the

donor bone. Complications with its use may occur in as many as 25-30% of patients and 10-40% of patients do not achieve a solid fusion (14, 81, 29). The morbidity of harvesting autograft has been well documented and includes chronic donor-site pain, infection, neurologic injury, blood loss, cosmetic deformity, bowel injury, vascular injury, hernia, and prolonged surgical and hospitalization time (27). Sasso *et al* reported persistent pain lasting at least 2 years after surgery in 15-39% of patients (81).

In addition, multilevel fusions often require substantial amounts of graft material and can exceed the available amounts in the iliac crest. Thus, the quantity of bone available to harvest may be insufficient for a long, multisegmental fusion or in a patient with previous graft harvest. Instrumentation to the pelvis can also prohibit the extent to which the crest can be used without loss of fixation (27).

Eliminating iliac crest bone graft harvest has the potential of decreased blood loss, decreased surgical time, and earlier hospital discharge, which, in combination with early, stable bone union and elimination of donor site comorbidity should result in better clinical outcomes. A search for a true autograft substitute has been an active area of research for at least 20 years. Multiple choices for graft alternatives have been introduced into the market. These graft alternatives have undergone varying degrees of regulatory scrutiny, however, none have level 1 evidence to support their efficacy in adult scoliosis surgery (14).

Allograft

Allograft is the second most commonly transplanted tissue, secondary only to blood (47). Allograft is bone taken from a human cadaver and transplanted into the fusion site. Allograft is osteoconductive and may also be osteoinductive depending on its preparation (66). Allograft

bone from cadaver donors requires meticulous attention to donor selection criteria. A comprehensive sociomedical history must be obtained, the cause of death determined, and serologic and other laboratory testing performed. The bone has to be harvested under sterile conditions within the first 12-24 hours after death, cultured, and processed for preservation and storage (14). Allograft is nearly unlimited in quantity, it saves operative time and cost associated with harvesting of autograft, and avoids donor site morbidity (27). In addition, allograft bone does not require human leukocyte antigen (HLA) cross-matching because most of the antigenic stimuli are removed during the processing, sterilization, and preservation procedures (27, 76, 80). Immunogenicity and the maintenance of the osteoinductive and osteoconductive properties of the allograft bone are related to the method of graft processing and preservation (66).

Disadvantages of allograft include the potential risks of bacterial contamination and possible transmission of viral diseases such as those caused by the human immunodeficiency virus (HIV) and hepatitis virus (66). The rate of HIV transfer from allograft bone is estimated at less than 1 in 1 million (27, 90). In addition, the processing and sterilization procedures can lead to other changes in graft properties, such as loss of osteogenic properties, decreased mechanical strength, decreased osteoinductive potential, delayed time of fusion, and potential nonunion (14, 82).

Although there are many animal studies evaluating allograft, few clinical studies of adequate design have been reported. Products considered to involve minimal manipulation of cells or tissues are regulated as tissue rather than devices. As a result no standardized burden of proof level for safety or effectiveness is required before these products are marketed and used in human patients (13, 17). In general, allograft has compared favorably with autogenous bone in interbody fusions. Dodd *et al* and Aurori *et al* recommend using allograft in spinal interbody

fusion surgery for scoliosis (36, 7). Both groups reported high fusion rates for allograft fusion without the morbidities associated with iliac crest bone graft harvest. However, results have not been reliable when allograft was used posteriorly, where it incorporated slower and less completely with decreased vascularization in comparison to autograft (40). Jorgenson et al and An *et al* concluded that allograft alone were not able to achieve a sufficient fusion rate for posterior spinal fusion in adult patients (5, 54). Therefore, allograft bone on its own is generally unreliable for stimulating fusion without the contribution of added factors.

Bone Marrow Aspirate

Aside from autogenous bone graft, bone marrow is the only other readily available source of osteoprogenitor cells. Marrow is, in fact, the bioactive component of autograft and has been regarded as a rich source of osteoprogenitor cells (87). The addition of bone marrow aspirate (BMA) has been shown to enhance the performance of a wide variety of bone graft materials (68, 73). The osteogenic potential of bone marrow used for grafting is dependent on the number of viable osteogenic precursor cells, or Mesenchymal stem cells (MSCs), placed into the operative site (87). Iliac crest bone marrow contains the highest percentage of osteogenic cells, and in a young patient, approximately 1 per 50,000 nucleated bone marrow cells is an osteoprogenitor cell (47). The marrow is harvested by needle aspiration from the patient's pelvis, which is a relatively nonmorbid procedure (68). After harvest, the aspirate is combined with an osteoconductive material. The osteoconductive scaffold provides the framework for the migration and attachment of the MSCs and the environment necessary for the synthesis of the extracellular matrix (82). By adding MSCs to the osteoconductive scaffold the composite graft becomes osteogenic, in effect providing a competitive alternative to autograft (39). Bone marrow aspirate with HEALOS® (DePuy Spine, Raynham, MA), a collagen-hydroxyapatite

sponge (CHS), was approved by the Food and Drug Administration (FDA) in December 2001 as a bone graft substitute (4).

In preclinical trials, Tay *et al* demonstrated that in posterolateral lumbar fusion (PLF), CHS soaked in BMA produced fusion rates comparable with those produced by autologous graft (87). In human clinical trials, BMA on CHS used in single-level and multi-level posterolateral lumbar fusion showed no significant difference when compared to ICBG (10, 25, 39, 41, 74). In multilevel posterolateral lumbar fusion, Neen *et al* reported equivalent fusion rates for the two groups, while BMA fusion was lower in lumbar interbody fusions, reporting a 20% fusion failure (74). Carter *et al* observed 95% fusion in multilevel TLIF/PLF when using BMA/CHS, concluding that BMA/CHA can be considered a bone graft substituted when used in TLIF/PLF (25). Studies have also confirmed the histological and mechanical properties of the fusion mass produced by BMA/CHS to be adequate for posterior fusion (10, 41). Therefore, deformity surgeons have been using Bone Marrow Aspirate (BMA) combined with HEALOS®, a collagen-hydroxyapatite sponge (CHS), in patients requiring fusion of multiple levels.

Nevertheless, BMA use is challenged by a range of problems including inconsistent or low concentrations of endogenous osteoprogenitor cells. Muschler *et al* demonstrated the influence of aspiration technique and volume on the concentration of MSCs. The study documented rapid dilution of MSCs by peripheral blood as aspiration volumes increased from one cc to four cc (68). Thus volume of the aspirate has been limited to less than 2 cc per site to reduce dilution. In addition, BMA may be less effective in patients who are experiencing a decline in their mesenchymal stem cell repository as a result of age, systemic illness, or osteoporosis. Several studies have published data on the age-related decline in the number and activity of MSCs (72, 82, 85). Moreover, due to the high degree of inter-individual variation in the osteoprogenitor cell

concentration of bone marrow, the osteogenic capacity of BMA may vary (82). BMA containing less than 1000 MSCs/cc has been shown to be ineffective for arthrodesis although the optimal number of cells required in a graft site is not known (53). Therefore, fresh bone marrow that has not undergone any treatment or culturing may not be as effective at promoting osteogenesis.

Several techniques have been developed to concentrate bone marrow derived MSCs. Different scaffolds have been developed with specific characteristics and biocapabilities that increase MSC adherence. By decreasing the flow rate of bone marrow through the matrix, its surface properties facilitate the rapid attachment of MSCs and removing red blood cells, serum and most other marrow components (69). This process could be carried out perioperatively and does not require expensive instrumentation. Another technique, cellular expansion of bone marrow derived MSCs in vitro, effectively concentrates the cells through cellular engineering, producing unlimited numbers of MSCs (46). However, the procedure takes several weeks, requires two separate surgeries and sophisticated instrumentation, and is expensive. In addition, there are risks of contamination and mutations (58, 69). This procedure is not approved, has not been tested in humans, and does not seem to have much future potential.

Bone Morphogenetic Protein (BMP)

Dr. Marshall Urist discovered bone morphogenetic proteins in 1965 while observing the capacity of demineralized bone to induce ectopic bone formation in a rat muscle pouch (93). Urist introduced the concept that bone growth factors can induce new bone formation independent of the bone tissue environment. Subsequently, bone morphogenetic proteins (BMPs) were isolated from demineralized bone. To test for their osteoinductive potential, BMPs were placed beneath the skin of test animals to assay for bone formation (89).

BMPs are found within the bone in small quantities. BMPs are part of the transforming growth factor β -superfamily (TGF β) and have an important role in bone formation, fracture healing, and repair of other musculoskeletal tissues (26, 49, 63). BMPs are growth factors that naturally exist within the bone matrix and act as pleiotropic regulators of chemotaxis, mitosis, differentiation, stimulation of extracellular matrix synthesis, binding to matrix components, maintenance of phenotype, and apoptosis (93). In addition, BMPs regulate bone volume.

Active BMPs induce bone formation in vivo in a stepwise fashion. The key steps are chemotaxis, proliferation, and differentiation of pluripotent mesenchymal cells (12, 14). BMPs rely on the host bed to provide the MSCs. Several local growth factors positively influence the migration and activity of potential bone forming mesenchymal stem cells (MSCs) from the surrounding tissues toward an osteoconductive matrix. BMPs interact with specific receptors on the cell surface of MSCs, initiating proliferation and differentiation of MSCs according to their environment (93). BMPs are the only growth factors that can stimulate differentiation of mesenchymal stem cells into the osteoblastic direction (49). BMPs may also stimulate the synthesis and secretion of other bone and angiogenic growth factors.

Bone morphogenetic protein comprises only 0.1% by weight of all bone protein (14). To provide reproducible amounts of isolated human BMP, it must be manufactured using recombination. The recombinant form of BMP, recombinant human bone morphogenetic protein (rhBMP) is identical to the natural form in both its chemistry and its ability to heal bone. Since BMPs are soluble and can diffuse away from the fusion site easily, they are combined with a carrier matrix that serves to retain the concentration and release BMPs consistently over time (66). On 2 July, 2002, the Food and Drug Administration (FDA) approved the use of rhBMP-2 (recombinant human bone morphogenetic protein-2, INFUSE TM, Medtronic Sofamor Danek,

Memphis, TN) for single level anterior lumbar interbody fusion with a titanium stand alone LT-cage (Medtronic Sofamor Danek, Memphis, TN) (3). The InFUSE bone graft consists of rhBMP-2 and a bovine type-I collagen carrier for the protein. Combined with the cage, the InFUSE bone graft has only been approved and demonstrated safe by the FDA for anterior lumbar interbody fusion. The cage is intended to maintain spacing within the spine while the graft is intended to form bone for spine stabilization.

Several studies have evaluated the use of BMP in single-level anterior interbody fusion and have concluded that the fusion rate for BMP is comparable to autograft while eliminating adverse clinical outcomes. A decrease in surgical time, blood loss, and hospital stay was observed in the groups treated with BMP when compared to autograft. The investigators concluded that BMP is a viable alternative for autograft in anterior fusion and may even produce higher fusion rates (15, 19-24).

Even though BMP has only been approved for the use in single-level anterior interbody fusion, several clinicians have used BMP in posterior fusion off-label. Several studies have evaluated fusion rates and outcomes using BMP in posterior lumbar spine fusion. While the fusion rate was high, reaching up to 100%, the concentration and amount of BMP used is higher compared to anterior fusion (13, 33, 34, 45). Several investigators have voiced their concern regarding the high concentration used. Glassman *et al* and Dawson *et al* used BMP at lower concentrations and were able to achieve relatively high fusion rates (95%). Both studies showed fusion success despite lower concentration (31, 43). Multilevel anterior and posterior fusion with BMP showed successful fusion rates without any complications as well (44, 51, 61, 67). Two studies showed a higher rate of complications when BMP was used in posterior lumbar interbody fusion. In both studies, heterotrophic bone was formed extending into the spinal canal. Though

no clinical symptoms were correlated with this adverse event, both studies were suspended and the investigators no longer use BMP in posterior lumbar interbody fusion (50, 65). Other complications and adverse events were reported when BMP was used in the cervical region. BMP produced higher nonunion rates, possible due to BMP-induced differentiation of osteoclasts (62, 78, 88). In addition, several studies observed swelling, difficulty breathing, and heterotrophic bone formation when BMP was used in the anterior cervical spine (30, 77, 83, 91, 92). On July 1, 2008, the FDA released a public health notification alerting healthcare providers of life-threatening complication associated with BMP usage in cervical spine fusion (1).

Thus, while BMP shows good results in certain applications, its usage must be evaluated carefully. In anterior interbody fusion, BMP has had high fusion rates without the morbidities associated with bone graft harvesting. However, in posterior fusion the concentration of BMP used currently is high and adverse effects may not be observable yet.

Overview of Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are non-hematopoietic, stromal cells that exhibit multilineage differentiation capacity being capable to give rise to diverse tissues, including bone, cartilage, adipose tissue, tendon, and muscle. MSCs reside in diverse host tissues (52, 58); however, bone marrow aspirates are considered to be the most accessible and enriched source of MSCs. Mesenchymal stem cells are capable of responding to their environment to differentiate into a variety of cells as needed (58, 70). When implanted in patients, MSCs and osteoprogenitor cells do not stimulate an immune response because they do not express immunologically relevant cell surface markers (76, 80). In pre-clinical trials with large animal models, allogeneic MSC

implants between genetically mismatched individuals have been shown to strongly enhance arthrodesis without the use of immunosuppressive therapy (6, 86).

Numerous research groups are currently studying the use of MSCs, with or without genetic modification, to enhance spinal fusions. Muschler, *et al* demonstrated that concentrated marrow aspirates can improve the efficacy of spinal fusions in a canine model (73). Prospective human clinical studies are needed to validate the use of bone marrow stem cells in posterolateral lumbar fusion.

Specific Aims

The primary goal of this study was to compare the use of bone marrow aspirate with the use of bone morphogenetic protein (rhBMP-2) in multilevel adult spinal deformity surgery. This was accomplished using data from a retrospective study at a single site of primary surgical patients with adult deformity who underwent anterior correction with a minimum 2 year follow up. I assisted in drafting the manuscript for this particular study.

The secondary goal of this study was to examine the feasibility of studying adult stem cells as a grafting alternative in adult spinal deformity surgery. This was done by doing extensive background literature research to find a product which could be used for this purpose. I drafted a protocol for the use of this stem cell product in spinal fusion and submitted an application to the Baylor Research Center Institutional Review Board (IRB).

Specific Aim 1: To evaluate the extent of fusion using bone marrow aspirate (BMA) obtained from iliac crest versus bone morphogenetic protein (rhBMP-2) in adult scoliosis patients undergoing anterior lumbar interbody fusion surgery.

Hypothesis: BMA will have the same rate of fusion when compared to rhBMP-2 in patients undergoing spinal fusion surgery.

Specific Aim 2: To develop a protocol to determine the feasibility of studying the use of adult stem cells in spinal fusion surgery for adult scoliosis.

Hypothesis: This protocol will be suitable for a study of the efficacy of adult stem cells in spinal fusion surgery for adult scoliosis.

Specific Aim 3: To analyze the difficulties and problems encountered in designing a protocol for studying various biologics used in spinal fusion surgery.

Hypothesis: This report will analyze some of the problems associated with studying spinal fusion biologics.

Significance

Scoliosis is a disorder of the spine causing the spine to curve laterally. As the spine curves abnormally, the involved vertebrae are forced to rotate creating a rib hump. In patient with scoliosis, the shoulders or pelvis may not be level, or waist asymmetry may be noted. In addition to visual deformity, untreated scoliosis in the adult can lead to painful spinal osteoarthritis, progressive deformity, spinal stenosis with radiculopathy, and muscle fatigue (16).

Approximately 12 million people are affected by scoliosis worldwide. Treatment may include spinal fusion, which is the joining or fusing of one or more vertebrae to reduce pain and stabilize the spine (17, 32). To help achieve this biological fusion, bone grafts or other biological

products that promote bone growth must be used. Currently there are many different types of biologics that surgeons can use to help attain solid spinal fusions.

It is estimated that approximately 500,000 bone graft procedures are performed in the United States each year. This potentially represents a one to two billion dollar per year market for the use of bone repair enhancers or bone graft substitutes (11). In 2006, over 349,000 spinal fusion procedures were performed. The mean age of patients getting spinal fusion was 53 years (2). Nevertheless, fusion failure occurred in 5% to 35% of cases (14, 27). New surgical techniques and fusion procedures may decrease this rate. The current study aimed to evaluate some of the common spine graft substitutes. In addition, the feasibility of implementing an investigational, randomized clinical trial using Mesenchymal stem cells was evaluated.

Materials and Methods

Manuscript for HEALOS study

In order to familiarize myself with the subject at hand I conducted a literature search using PubMed with the following keywords: Scoliosis, Spinal Fusion, arthrodesis, HEALOS, Bone Marrow Aspirate (BMA), Bone Morphogenetic Protein-2, pseudarthrosis, Mesenchymal stem cells, bone graft substitutes, bone graft replacement, bone regeneration, adult scoliosis, multilevel fusion, SRS-22, iliac crest bone graft. I then had a discussion with Mr. Eric Buchl, the research Physician Assistant at the Scoliosis Center, about the paper and the data to be presented.

Over the following few weeks I worked on the manuscript while also observing various aspects of the clinical research setting. I submitted a rough draft to Mr. Buchl and subsequently met with him to discuss revisions and possible problems. For the materials and methods section I

needed more information, but I nevertheless wrote a rough draft for the section. After two more drafts, Mr. Buchl submitted the paper to be reviewed for the upcoming Scoliosis Research Society (SRS) convention at which he presented the paper.

Mesenchymal Stem Cell Product

I searched the internet for a product for my Mesenchymal stem cell (MSC) project. The ideal product would contain allogeneic MSCs that are indicated for the use in spine fusion surgery. I found a new product by an Australian biotechnology company, Mesoderm, Inc. I contacted the company representative to get some more information about the product and did some additional research about the product. It was an “off-the shelf” Mesenchymal stem cell product from a single donor. MSCs lack certain cell surface markers allowing them to elude T-cell mediated cell rejection and therefore are immune-privileged (76, 80). It is therefore possible to use allogeneic MSCs without having to worry about rejection. Unfortunately, the company was not able to give me information that was pertinent for me to receive Institutional Review Board (IRB) approval. Since the product was not on the market and not approved by the FDA, I needed the Investigational New Drug approval number in order to conduct human research.

I then continued my search for another product. I found an allogeneic product that would be satisfactory for my needs. Trinity® Evolution™ is a minimally manipulated bone graft substitute, a cancellous bone allograft matrix containing viable adult mesenchymal stem cells and osteoprogenitor cells as well as a demineralized bone component to promote bone growth. Trinity® Evolution™ is an off-the-shelf bone grafting product. It is a minimally-manipulated, human cellular and tissue-based allograft product that has undergone selective depletion of immunogenic cells, leaving stem cells with demonstrated multipotentiality, but with negligible

risk of inflammation or immune response. Trinity® Evolution™ is extensively tested for consistently high concentrations of bone-forming mesenchymal stem cells (MSCs). The role played by the osteoprogenitor cells in Trinity® Evolution™ is similar to the role these cells play during natural bone repair. Upon implantation, the adult MSCs act as though part of the patient's own body, differentiating into the specialized bone cells called on for healing at the surgery site. Since Trinity® Evolution™ provides all three bone growth properties - osteoconduction, osteoinduction, and osteogenesis - it offers all the benefits of autograft without the risks or discomfort during patient recovery associated with the graft donor site.

Trinity® Evolution™ is regulated by the US Food and Drug Administration (FDA) as Human Cells, Tissues, and Cellular and Tissue based Products (HCT/Ps) under 21 CFR part 1271, and meets or exceeds all applicable standards, which include donor screening, processing, process controls and validation, storage, and recordkeeping.

While the product had all of the necessary characteristic for an ideal bone graft substitute, it still posed the risk of infection. In addition, the manufacture does not mention if the graft is enriched in mesenchymal stem cells or if the cells present are simply bone marrow stem cells of the original graft. However, this was the only product that would be feasibly for my study at this time.

Protocol and IRB Submission

I drafted a protocol to study the extent of fusion and clinical outcomes using Trinity® Evolution™ in Anterior lumbar Interbody Fusion (ALIF) with posterior instrumentation in adult scoliosis patients. I also wanted to compare my results with fusion rates and clinical outcomes obtained by using Bone Marrow Aspirate.

I chose the study to be Pilot study due to the small sample size of 40 patients for each group and after having a discussion with the IRB. The study was to be a randomized, parallel group, prospective study in patients diagnosed with adult scoliosis, who have elected surgical treatment. Qualifying patients would be randomized, using simple randomization tables, to either of two groups. In Group 1, patients will undergo spinal fusion using the investigational product in a cage in an ALIF procedure with posterior instrumentation. In Group 2, patients will undergo spinal fusion using BMA in a cage in the same procedure.

The evaluation of subjects includes pre-op radiographs and questionnaires; 12 month follow-up and 24 month follow-up CT scans, two-view scoliosis x-rays, and flexion-extension radiographs. Analysis of these radiographic images will be used to determine fusion status. Established research questionnaires including the Visual Analog Scale (VAS), Oswestry Disability Index (ODI), Scoliosis Research Society-22 (SRS-22), and SF-36 will also be analyzed.

I prepared the protocol using prior studies implemented at the Scoliosis Center as a template, such that fusion success is defined by both radiographic and clinical success. Assessment of radiographic fusion success and clinical fusion success are defined using the following guidelines:

Radiographic Fusion Success

Fusion will be assessed by an independent radiology review at the pre-op, 12 and 24 month visit. Follow-up CT scans, two-view scoliosis x-rays and flexion-extension radiographs will be analyzed by an independent blinded orthopedic deformity surgeon and a neuroradiologist. The Lenke Classification of posterolateral Fusion will be used to grade the fusion success (18):

- Grade A: Definitely solid with bilateral trabeculated stout fusion masses present
- Grade B: Possibly solid with a unilateral large fusion mass and a contralateral small fusion mass.
- Grade C: Probably not solid with a small fusion mass bilaterally
- Grade D: Definitely not solid with bone graft resorption or obvious pseudarthrosis bilaterally
- Fusion success is defined as: Lenke “A” or “B”

Clinical Success

Patients will be evaluated for failure to meet minimal clinically important difference (MCID), which is defined by Buchl *et al* as:

- Greater than 15% improvement of preoperative SRS scores
- Greater than 10% improvement of preoperative ODI score

Additionally, the absence of Serious Adverse Events will be evaluated. Serious Adverse Events include:

- Revisions
- Reoperations
- Paralysis

- Death

Subject Inclusion Criteria

- Between and including the ages of 18 and 64 years
- Women may be of childbearing age but must not be currently pregnant or planning to conceive for the duration of the study
- Diagnosed scoliosis deformity confirmed by CT, MRI, or plain film
- Must be candidate for spinal fusion surgery
- Must not have history of drug or alcohol abuse within 1 year of study enrollment
- Capable of providing informed consent
- Subject must be willing to adhere to all study requirements and follow up visits as necessary

Subject Exclusion Criteria

- Subjects with infection, malignancy, enteric contamination in the operative site
- Infection in the disc or spine, past or present
- Subjects who have rheumatoid arthritis or autoimmune disorders

- Subject is immunocompromised or being treated with immunosuppressive agents
- Significant osteoporosis or metabolic bone disease
- Pregnant or lactating, or wishes to become pregnant within duration of study
- Any contraindicative medical conditions that may affect surgery or success of corrective spinal fusion
- Has a history of hypersensitivity or anaphylactic reaction to murine or bovine products, dimethyl sulfoxide (DMSO), or titanium
- Has a history of epidural steroid injections within 1 week prior to study treatment

After I prepared the protocol and informed consent, I filled out all of the IRB paperwork necessary for IRB submission. I submitted the protocol and IRB application for pre-approval. After reviewing my protocol, one of the IRB members asked me to clarify some several issues in my protocol, such as a better definition of the risks involved as well as some radiation risks. I made the appropriate changes and resubmitted the protocol and application.

I attended the full Review Board Meeting for my project and was there to answer any of the questions the board had regarding my project.

Results and Discussion

Results

The clinical trial was designed in order to assess the feasibility of implementing a randomized clinical trial using Mesenchymal stem cells in spinal fusion surgery. During the IRB Meeting, the board members seem to have several questions/objections including:

- There were several questions regarding the “standard of treatment”, which is very difficult to define for multilevel fusion surgeries. Even though iliac crest bone graft (ICBG) is the “gold standard” for bone grafting procedures, it is not very commonly used in scoliosis surgery. Due to the nature of multilevel fusion required for scoliosis correction, the amount of graft possible from ICBG is not sufficient and alternative grafts have been employed. At the Baylor Scoliosis Center, Bone Marrow Aspirate with a collagen sponge is used most frequently. It is for that reason I chose to compare the investigational product to BMA rather than ICBG. Nevertheless, the board felt that I should compare my investigational product to ICBG.
- There were also concerns regarding what would happen in the case of failure of fusion and how this would be addressed. I explained such a case in my protocol as well as my IRB application and also tried to explain it to the board when the question came up. In spinal fusion surgery the risk of non-union is present regardless of bone graft substitute. With better surgical technique and an ideal bone graft this risk will be eliminated. In the case of failure and the presence of clinical symptoms, such as pain and curve progression, it is necessary to re-operate.

- Concerns regarding radiographic radiation risks were also brought up. I used the same numbers for radiation risks that have been used in all of the previous Scoliosis Center protocols. I also checked with the radiology technician who performs the radiographs for the Scoliosis Center to validate the numbers. Nevertheless, one of the IRB members questioned the numbers and felt that they were larger than necessary.

Two weeks after the IRB meeting I received the decision. The board tabled my protocol and asked me to make several major modifications and resubmit. The following were changes were required:

- The FDA status for both treatments needed to be included
- The option of autogenous bone graft needed to be revisited
- A better definition of the randomization process
- Relative risks of failure of fusion needed to be included
- A definition of which treatments and procedures were for study purposes only and which were standard of care needed to be included
- A better definition of nonunion and consequences thereof
- Change radiation exposure and radiation risks

Discussion

While this protocol was not going to be implemented during my internship, the value of this study was to look at some of the problems that can arise when conducting research in spinal surgery. One of the first problems I ran into was a clear definition of standard of care. Because spinal fusion surgery is multifactorial, there is no clear definition of the standard of care for surgical technique or bone graft choice. Each surgeon has their own preference, which is mostly based on experience rather than sound scientific principles. This makes it very challenging to conduct research in this area in particular when more than one surgeon is operating. Thus differences in perioperative and postoperative care may impact the outcome.

In addition, because scoliosis surgery is not a very common procedure, the patient numbers are small and data obtained from clinical trials may be difficult to interpret. Also, executing a clinical trial in a surgical setting may pose additional problems such as blinding or placebo and recruiting willing subject. Randomization is difficult in surgical trials because a surgical procedure is permanent and subjects may not be willing to participate. Also, because inclusion/exclusion criteria are usually very strict for surgical clinical trials, the results may not be generalizable or applicable to all patients with the disease. The majority of clinical trials involving scoliosis surgery are very specific to the approach, technique, and patient sample. This adds to the problem of lack of standardization.

Finally, a number of studies in the medical literature have shown that there is a lag between the time of publication of credible scientific information that should change medical practice and the time of the adoption of its change by practitioners (64, 94). This may be particularly true in orthopedic surgery. Even with appropriate information, clinicians are reluctant to change their treatment. This may be due to the lack of well-designed, evidence based

clinical trials. In addition, clinicians usually wait until at least the 10-year follow-up before they consider using a procedure or device.

Summary and Conclusions

A well-designed, randomized clinical trial testing an investigational procedure or product in scoliosis spinal fusion surgery is difficult to implement and comes with a variety of difficulties. While the goal of this study was not to look at the outcomes of the clinical trial, it was an opportunity for me to look at various problems encountered in designing a clinical trial. In creating the protocol I became aware of how important it is to have a good understanding of the current literature in the particular field and to make sure the study is looking at a variable that will impact patient care in a way that will be significant to the clinician and patient. In addition, it is important to understand the various types of clinical trial designs and the impact each will have on scientific knowledge. A study analyzing data retrospectively may not be as influential as a randomized clinical trial. However, it is not always possible to conduct a randomized trial and it is therefore important to design the study to give the most evidence possible. I also became aware of how difficult it is to define standard of care. Since each surgeon had their own approach it is important to standardize procedures before beginning a trial.

I had the opportunity to go through all of the steps required to get IRB approval. This includes compiling the study packet with all of the necessary forms, signatures, and other paperwork. In addition, I attended the IRB meeting and was exposed to the regulatory aspect of clinical trials. While my protocol proved to have several problems and was not approved by the IRB, I gained valuable insight into some of the common difficulties in getting approval for a study. It is important to understand that the member of the IRB may not be very familiar with

your subject of study. Therefore, it is critical to have a very clear explanation of the different procedures and what the standard of care is. With better standardization the majority of these problems would be eliminated and lead to better, meaningful trials and comparisons.

CHAPTER III

INTERNSHIP EXPERIENCE

Internship Site

My internship site was at the Baylor Scoliosis Center located on the campus of Baylor Regional Medical Center at Plano, where I worked as a clinical research coordinator. Since 2008, the research department has conducted advanced research projects that strive to ultimately improve the quality of life for scoliosis patients and revolutionize the way scoliosis is treated. The goal of the Scoliosis Center is to become a premier scoliosis research center internationally. Currently, pain, spine fusion, genetics, possible scoliosis biomarkers, surgical techniques, instrumentation, and surgical outcomes are being investigated in nine active studies. In addition, as a result of exceptional research efforts, the work of the Scoliosis Center has been presented at several international conferences.

In 2009, the Baylor Scoliosis Center opened a satellite clinic on the campus of Baylor All Saints Medical Center at Fort Worth to better serve patient throughout Texas. In addition to the clinical expansion, the research department has several possible new projects waiting. The clinicians and the research team work closely together to get the best data possible for their studies.

Journal Summary

At the beginning of my internship I spent a lot of time doing background research and reading journal articles. Because Scoliosis is a condition of which I knew little about and has many different aspects associated with it, I had a lot of information to absorb. In addition to working on my practicum report I was also able to work closely with the research team on the current study protocols. I looked at the different study binders and organized some of their content. In addition, I was able to observe the clinical team in clinical and surgical settings. I was able to assist with the preparation of IRB (Institutional Review Board) documents. I did the revisions for several trials to change study personnel, add Baylor All Saints as a study site, and change some of the procedures. I also did some of the continuing review paperwork for several of the studies.

In addition, I assisted with case report form completion and subject de-identification. I observed the study staff consent patients and answer their questions as well as coordinate their appointments. I watched data collection and verification procedures and the interaction with different study sites on the same protocol for multi-center studies.

In addition, I assisted in drafting the manuscript for one of the studies as well as designing a poster presentation. The manuscript and poster were presented at the Scoliosis Research Society Conference in San Antonio. I was able to attend this conference and watch the presentations. In addition, at the conference, we attended a meeting with one of the groups the Scoliosis Center conducts research with. The International Spine Study Group (ISSG) focuses mainly on clinical outcomes and risk/complications. I observed the various surgeons interact and come up with ideas for new studies.

I also attended the research meeting at the Scoliosis Center conducted every other Tuesday morning. During these meeting, the research team and clinical team discuss any current problems and talk about recent changes. In addition, projects to be completed are discussed and any problems are looked at. On opposite Tuesday mornings, I attended surgical case conferences at the hospital. During these meetings, the different surgical cases for the following two weeks were presented to the various providers that will be involved in the care of the patient. I also assisted in making some of the power point presentations for these meetings.

This internship also offered several additional classes and educational opportunities. I attended several classes that taught me how to develop a study budget, implement a study, ship biologic material, and draw blood. I attended an IATA/DOT Packaging and Shipping of Hazardous Material class and became certified to package, fill out paperwork for, and ship biologic materials. I took several webinars through the Baylor Learning Network that focused on clinical research. I was also trained how to use StudyManager, a program that is intended to keep track of different research studies at individual sites. Information such as patient demographics, providers, and financial information can be added to the program. I also attended two classes conducted by MedTrials on regulatory guidelines and good clinical practices.

My complete Journal, outlining my internship experience can be found in the Appendix. I summarized some of the main events for each day.

APPENDIX

INTERNSHIP JOURNAL

Internship Journal

Suncica Hukic

Baylor, Plano

Week of 06/01/2009-06/05/2009

Monday 06/01/2009

- 8:00: checked in with Nanette and received my “New Employee Orientation Binder”
- 8:30 – 11:30: worked on Baylor Learning Network (BLN) IRB Modules which included 7 Modules:
 - o BRI: Definitions and Examples of Research (2009)
 - o BRI: Exemptions and Expedited Review (2009)
 - o BRI: Introduction to the IRB Process (2009)
 - o BRI: Principal Investigator Reporting Responsibilities (2009)
 - o BRI: Recruiting, Screening, Consenting and Retaining Research Subjects (2009)
 - o BRI: Risk Assessment in Research (2009)
 - o BRI: Special Considerations for Vulnerable Subjects (2009)
- 11:30 – 12:30: Lunch
- 1:00 – 2:00: went through the New Employee Orientation Binder with Nanette
- 2:00 – 4:30: searched for relevant Journal articles for my project and shadowed the Research Team which consists of Chantelle Freeman, Erin McCullough, and Kyndra Walker

Tuesday 06/02/2009

- 7:00: attended my first Research meeting with Dr. Hostin, Jay, Chantelle, Erin, and Kyndra.
 - o During the meeting Dr. Hostin showed us his presentation he will be giving to potential patients in Fort Worth.
 - o Dr. Hostin then addressed some issues he has with DCS (Dynamic Clinical Systems)
- 8:00 - 11:00: went through the Healos binder, which has subject information and the Protocol as well as any of the revision done on the Protocol over the period of the study
- 11:30 – 12:30: Lunch
- 12:30 – 4:00: searched for journal articles and did some research to get a better idea for my prospective project

Wednesday 06/03/2009

- 8:00 – 10:00: background research on scoliosis and the different surgical methods used for spinal correction
- 10:00 – 12:00: de-identified x-rays and CT scans for the Healos study
- 12:00 – 1:00: Lunch
- 1:00 – 5:00: continued to de-identify x-rays for Healos study

Thursday 06/04/2009

- 9:00 – 12:00: went to “StudyManager” Training; the program is intended to keep track of different research studies at individual sites including patient information, scheduling, procedures, providers, and financial information. The scoliosis center may implement this program to keep track of the different research subjects.
- 12:00 – 1:00: Lunch
- 1:00 – 5:00: continued to research scoliosis and different treatment types

Friday 06/05/2009

- 9:00 – 11:30: read journal articles
- 11:30 – 12:30: Lunch
- 12:30 – 4:00: de-identified x-rays and CT scans for the Healos study

Week of 06/08/2009-06/012/2009

Monday 06/08/2009

- 8:00 – 11:30: searched different journal articles
- 11:30 – 12:30: Lunch
- 12:30 – 4:00: de-identified x-rays and CT scans for the Healos study

Tuesday 06/09/2009

- 7:00 – 8:00: Surgical Case Conference for June 22nd through July 3rd
 - o The different surgical cases for the two weeks were presented
 - o Each patient was looked at individually and post-operative care was discussed
- 9:00am – 2:30: read different journal articles
- 2:30: went to Baylor (downtown) to get TB test administered

Wednesday 06/10/2009

- 8:30 – 9:30: attended meeting with Statisticians for Baylor
- 9:30 – 1:30: researched articles that looked at the different Scoliosis Research Society (SRS) Questionnaire and the correlation of the questionnaire with pseudarthrosis
- 1:30 – 2:30: Lunch
- 2:30 - 5:00: read articles pertaining to SRS questionnaires and the different mechanisms to identify pseudarthrosis

Thursday 06/11/2009

- 8:00 – 12:30: Started adding the Healos study and patients into Study Manager
- 12:30 – 1:30: Lunch
- 1:30 – 5:00: finished adding the Healos study into Study Manager and started adding the Shoulder study into Study Manager

Friday 06/12/2009

- 7:30 – 9:00: went back to Employee Health at Baylor, Dallas to get TB test read
- 9:00 – 10:30: attended BRI Orientation
- 11:30 – 3:30: finished with the Shoulder study in Study Manager
- 3:30: Fire Drill

Week of 06/15/2009-06/19/2009

Monday 06/15/2009

Chantelle out of the office

- 8:00 – 12:30: read different SRS studies
- 12:30 – 1:30: Lunch
- 1:30 – 3:00: continued reading journal articles and searching for additional articles
- 3:30: went to Baylor downtown to pick up TB Report

Tuesday 06/16/2009

- 7:00 – 8:30: Research Meeting
 - o Discussed the different studies and what is missing
 - o Looked at new software
 - o Discussed what needs to be done
- 9:00 – 12:30: continued journal article search
- 12:30 – 1:30: Lunch
- 1:30 – 4:00: read journal articles

Wednesday 06/17/2009

- 8:00 – 11:30: Revised Informed Consent Forms for the Healos study and the ISSG study and changed Form 1. In addition filled out Form 7 explaining the changes being made and why the changes are being made
- 12:00 – 1:00: Lunch
- 1:00 – 4:00: deidentified x-ray and CT-scans for the Healos study

Thursday 06/18/2009

- 9:00 – 10:00: Meeting with Nanette and Chantelle to discuss my progress and talk about my thesis proposal
- 10:00 – 11:00: looked at thesis proposal requirements
- 1:00 – 4:00: IRB meeting at BUMC (downtown)
 - o First the board went over the continuing review protocols for about 1 hour
 - o The remainder of the time was spent on new projects and protocols. There were about four new protocols that were discussed. One of them was “tabled”, requiring some changes, two of them were “approved” with minor changes, and one of them was “approved” as is

Friday 06/19/2009

- 7:00 – 2:00: Observed Dr. Shelokov in surgery
 - o Surgery was spinal fusion surgery for scoliosis on a 31 year old male
 - o Dr. Shelokov did a posterior spinal fusion from T2-L3

Week of 06/22/2009 – 06/26/2009

Monday 06/22/2009

- 8:00 – 4:00: Shadowed Dr. Shelokov in clinic
 - o Dr. Shelokov saw about 17 patients for various reasons all related to scoliosis
 - o Some patients were follow-ups after surgery
 - o Dr. Shelokov had several new patients that came in to see him after being referred to him by their primary care physicians
 - o Several of the patients drove a long way to see Dr. Shelokov
 - o Dr. Shelokov has a very different approach to patient care; he takes his time to address any questions and concerns his patients have. He also talks to patients about other problems they may have and tries to help them in any way he can

Tuesday 06/23/2009

- 7:00 – 8:00: Surgical Case Conference
- 8:00 – 2:00: Looked at different articles that discuss gene therapy for spinal fusion. Eric came and talked to me about what he wants me to do regarding the article for the SRS conference
- 2:00 – 3:00: Talked to Nanette and Chantelle about my approach for my thesis. We laid out a plan for me to work on
- 3:00 – 4:00: Training for Hazardous Materials Shipping

Wednesday 06/24/2009

- 7:00 – 11:00: Helped Chantelle revise Protocol paperwork that needs to be turned in to the IRB for extended review
- 11:30 – 12:30: Lunch
- 12:30 – 3:30: Searched Journal articles that will be used for the Healos study

Thursday 06/25/2009

- 7:00 – 3:00: Read Journal articles that I will need for preparing the Healos study article

Friday 06/26/2009

- 7:00 – 3:00: Searched for Journal articles that I will need for my Research Proposal

Week of 06/29/2009 – 07/3/2009

Monday 06/29/2009

- 7:00 – 3:00: Continued to read and search for Journal articles for Research Proposal
- Helped Chantelle to make copies for new/approved Informed Consent forms for the different studies

Tuesday 06/30/2009

- 7:00 – 3:00: Worked on Research Proposal

Wednesday 07/01/2009

- Sick

Thursday 07/02/2009

- 7:00 – 3:00: Worked on Research Proposal; Finished writing the proposal and e-mailed it to my committee members

Friday 07/03/2009

- July 4th Holiday

Week of 07/6/2009 – 07/10/2009

Monday 07/6/2009

- 7:00 – 2:00: Worked on Journal article
 - o Read articles and worked on putting together Journal article
 - o Watched Erin Consent a patient for a study

Tuesday 07/7/2009

- 7:00 – 2:00: Worked on Journal Article
 - o Talked to Eric about the study and any questions I have

Wednesday 07/8/2009

- 7:00 – 3:00: Continued to work on rough draft for the manuscript

Thursday 07/09/2009

- 7:00 – 3:00: Continued to work on rough draft for manuscript which needs to be turned in tomorrow Friday before Eric leaves town

Friday 07/10/2009

- 7:00 – 3:00: finished rough draft for manuscript and turned it in
 - o Talked to Nanette and Chantelle about my project
 - o Started on literature review for my project

Week of 07/13/2009 – 07/17/2009

Monday 07/13/2009

- 8:00 – 3:00: Phlebotomy Class at BUMC

Tuesday 07/14/2009

- 7:00 – 3:00: Literature search for mesenchymal stem cells and spinal fusion

Wednesday 07/15/2009

- 7:00 – 3:00: Worked on Manuscript edits
Continued with literature search

Thursday 07/16/2009

- 7:00 – 3:00: Continued working on Manuscript edits and e-mailed Eric

Friday 07/17/2009

- 7:00 – 3:00: Looked for possible products that I could use for my project
Found a company that has a product that would work for my project
and read about the company and the product
- Meeting with Nanette and Chantelle to discuss progress

Week of 07/20/2009 – 07/24/2009

Monday 07/20/2009

- 7:00 – 3:00: Looked for information about Mesoderm (company that has a possible product for my project)
- E-mailed Mesoderm Company

Tuesday 07/21/2009

- 9:00 – 10:30: Clinical Research Coordinators meeting at BUMC
- 11:00 – 4:00: Observed Dr. Shelokov in surgery

Wednesday 07/22/2009

- 7:00 – 3:00: Worked on Thesis project
 - o Talked to representative at Mesoderm
 - o Looked up more information on Mesoderm

Thursday 07/23/2009

- 7:00 – 3:00: Worked on Project
 - o Did some more background research

Friday 07/24/2009

- 7:00 – 2:00: Worked on Project
- Meeting with Nanette and Chantelle

Week of 07/27/2009 – 07/31/2009

Took week off to prepare for MCAT

Week of 08/03/2009 – 08/07/2009

Monday 08/03/2009

- 8:00 – 5:00: Did literature search for Thesis project
 - o Worked on adding SRS-22 and ODI scores for patients into the Scoliosis Center Database

Tuesday 08/04/2009

Office closed

Wednesday 08/05/2009

Office closed

Thursday 08/06/2009

- 8:00 – 5:00: Added Clinical Outcomes Scores to the Database

Friday 08/07/2009

- 8:00 – 3:00: Worked on Thesis; did literature search

Week of 08/10/2009 – 08/14/2009

Monday 08/10/2009

- 8:00 – 5:00: Worked on Thesis
Worked on Manuscript edits

Tuesday 08/11/2009

- 7:00 – 8:00: Research Meeting
- 8:00 – 1:00: Worked on Thesis
- 1:00 – 2:00: Employee Town Hall Meeting
- 2:30 – 3:00: Weekly meeting with Nanette and Chantelle
- 3:00 – 4:00: Sat in on meeting with Nanette and Chantelle to discuss IRB form modifications

Wednesday 08/12/2009

Office closed

Thursday 08/13/2009

- 9:00 – 5:00: Work on Thesis Project and Manuscript

Friday 08/14/2009

- 8:00 – 3:00: Worked on Thesis Project

Week of 08/17/2009 – 08/21/2009

Monday 08/17/2009

- 9:00 – 5:00: Worked on Thesis Project
 - o Worked on Poster presentation for SRS

Tuesday 08/18/2009

- 9:30 – 11:00: Clinical Research Coordinators meeting at BUMC

Wednesday 08/19/2009

- 10:00 – 5:00: Worked on Thesis Project; prepared IRB documents

Thursday 08/20/2009

- 8:00 – 5:00: Worked on IRB documents for the submission of Thesis Project Protocol to the IRB

Friday 08/21/2009

- 9:00 – 10:00: Scoliosis Research Team meeting to discuss new procedures
- 10:00 – 4:00: Worked on IRB submission

Week of 08/24/2009 – 08/28/2009

Monday 08/24/2009

- 7:00 – 5:00: Worked on IRB submission for pre-review
 - o Problem: do not have IND number for product
 - o Spoke to Elizabeth, IRB director; I will need both IND number and investigational product brochure
 - o E-mailed company which makes the product to see if I can get all of that information from them
 - o E-mailed FDA to see if I can get some information from them
 - o E-mailed Texas Back Institute as they are doing a study with NeoFuse

Tuesday 08/25/2009

- 8:00 – 5:00: Looked at other possible products that I could use for my project in case I do not get all the information for NeoFuse, such as IND and the investigational product brochure
 - o Received e-mail form FDA and Texas Back Institute; they are not able to disclose any information to me

Wednesday 08/26/2009

- 8:00 – 4:00: Re-wrote protocol using Trinity® Evolution™ instead of NeoFuse
 - o Prepared all the documents to submit to IRB
 - o Submitted all documents and protocol to IRB for pre-review

Thursday 08/27/2009

- 8:00 – 4:00: Did BLN module on Research Billing Compliance
 - o Worked on surgical case conferences

Friday 08/28/2009

- 9:00 – 10:00: Research Billing Compliance Q&A Session
- 10:00 – 11:00: Weekly meeting with Nanette and Chantelle
- 11:00 – 3:00: Worked on surgical case conferences

Week of 08/31/2009 – 09/04/2009

Monday 08/31/2009

- 8:00 – 5:00: Worked on Thesis

Tuesday 09/01/2009

Sick

Wednesday 09/02/2009

- 8:00 – 4:00: Continued to work on Thesis; getting some more background information on Mesenchymal stem cells
 - o Watched as Erin took Clinical Photos for pre-op

Thursday 09/03/2009

- 8:00 – 4:00: Did literature search for more information for Thesis

Friday 09/04/2009

- 8:00 – 3:00: Weekly meeting with Nanette and Chantelle
- Received e-mail response from IRB regarding pre-review protocol with several questions and changes that need to be made:
 - o Need to change either number of subjects or change study to pilot trial
 - o Need to clarify wording regarding the “gold standard” for graft
 - o Need to clarify x-ray and CT imaging numbers
 - o Need to clarify risks

Week of 09/07/2009 – 09/11/2009

Monday 09/07/2009

Labor Day

Tuesday 09/08/2009

- 7:00 – 8:00: Research Meeting
- 8:00 – 3:00: Worked on modifying protocol for IRB submission

Wednesday 09/09/2009

- 9:00 – 5:00: Continued to work on modifying the protocol
 - o Changed study to pilot study
 - o Changed to parallel prospective study rather than matching patient cohorts
 - o Changed risks in both the protocol and informed consent form

Thursday 09/10/2009

- 9:00 – 5:00: Finalized changes for protocol and submitted the new documents for pre-review

Friday 09/11/2009

- 9:00 – 4:00: Worked on surgical case conferences

Week of 09/14/2009 – 09/18/2009

Monday 09/14/2009

- 8:00 – 4:00: Checked Isometric Shoulder Study spreadsheet for data accuracy and added missing data

Tuesday 09/15/2009

- 7:00 – 4:00: Worked on some additional changes that needed to be made to protocol; rearranged the layout and added some additional references

Wednesday 09/16/2009

- 8:00 – 4:00: Worked on spreadsheet for shoulder study

Thursday 09/17/2009

- 7:00 – 5:00: Finalized all the paperwork needed for the IRB submission; took several forms to Jerri Garison, President of BMC At Plano for her signature

Friday 09/18/2009

- 7:00 – 4:00: Picked up paperwork from Jerri Garison's office and got signatures from Dr. Hostin; prepared IRB New Study packets and dropped them off at the Baylor Research Institute Office
 - Form 1: Baylor Research Institute Institutional Review Board Application and Project Summary
 - The Protocol
 - Informed Consent
 - Form 18: Review of Scientific and Scholarly Validity

Week of 09/21/2009 – 09/25/2009

Monday 09/21/2009

- 9:00 – 5:00: Worked on Thesis outline and checked all the requirements for Thesis as well as the deadlines

Tuesday 09/22/2009

- 9:00 – 5:00: Worked on spreadsheet for shoulder study

Wednesday 09/23/2009

Worked from home on Thesis

Thursday 09/24/2009

- Scoliosis Research Society (SRS) Annual Meeting in San Antonio
 - o Listened to some of the presentations; Listened to Eric present the BMA vs. BMP paper
 - o Went to a meeting with DCS representative; they talked to Dr. Hostin and Eric about how the database can be improved and customized to make it easier for clinic
 - o Went to ISSG meeting; the physicians talked about ongoing studies and possible future studies
 - o Went to Axial Biotech Symposium, which addressed genetic testing for Scoliosis

Friday 09/25/2009

- Worked from home

Week of 09/28/2009 – 10/02/2009

Monday 09/28/2009

- 9:00 – 5:00: Worked on Thesis and setting the date to defend; looked at the exact formatting requirements for the thesis

Tuesday 09/29/2009

- 9:00 – 5:00: Added missing data to shoulder study spreadsheet and re-organized some of the tables; made sure all of the patient check requests have been submitted for the study dates
 - o Worked on putting parts of my thesis together

Wednesday 09/30/2009

- 9:00 – 5:00: Prepared everything for IRB meeting tomorrow; looked over my protocol and any possible problems that can be anticipated
 - o Went to presentation by Lynn Van Dermark (Medtrials) on FDA Audit Preparation Training including:
 - Investigator Responsibilities & Inspection Readiness
 - FDA Inspections
 - Preparing for an Inspection
 - Inspection Process

Thursday 10/01/2009

- 9:00 – 4:00: IRB Meeting; The IRB board had several questions/objections to my protocol
 - o One problem seems to stem from an apparent mix up of documents; it seems that the board did not get all of my updated forms because they had questions about the consent form quoting things from an old version that has been corrected
 - o There were several questions on the “standard of treatment”, which is difficult to define in multilevel fusion surgeries
 - o One member wanted me to clarify the differences between the two treatments and again clarify the standard of care
 - o Concerns regarding what happens in the case of failure of fusion and how this is addressed was also brought up even though this was stated and explained in the protocol
 - o Another concern was the cost and what will be paid by the study versus the insurance or patient
- Overall I feel that the main reviewer did not get the updated forms or did not read the updated information because several of his concerns were clarified in the new versions
- It also shows that it is a challenge for scoliosis researcher to clarify what the standard of care is because there is no set definition on this issue. In addition, there is not a clear agreement on what is considered success vs. failure of fusion and how to deal with this in the best way
- This was a very good experience and will allow me to try and explain/revise my thesis so that it is more clear as far as what the standard of care is

Friday 10/02/2009

- 9:00 – 5:00: Looked over my protocol and tried to see how I could address the problems indicated at the IRB meeting
- Worked on my Thesis

Week of 10/05/2009 – 10/09/2009

Monday 10/05/2009

- 9:00 – 5:00: Worked on Thesis
Added Study patient information for shoulder study for 1 year follow up into spreadsheet

Tuesday 10/06/2009

- 9:00 – 5:00: Worked on Thesis

Wednesday 10/07/2009

- went to Fort Worth to take Intent to Defend Form to Graduate Office
- worked on Thesis

Thursday 10/08/2009

- 9:00 – 5:00: went through a list of potential subjects screening them for the inclusion/exclusion criteria for a new study that looks at osteotomies; looked at patient files to make sure patient has none of the exclusion criteria; looked at surgical sheet to look for inclusion criteria.

Friday 10/09/2009

- 9:00 – 4:00: Worked on the revision for two studies. Made appropriate changes to the protocol, consent forms, and Form1. In addition, I filled out Form 7 for each study.

Week of 10/12/2009 – 10/16/2009

Worked on my thesis while I continued to observe the research team.

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