HEALTHY AGING RESEARCH

The Orthobiologic Institute (TOBI) 7th Annual PRP & Regenerative Medicine Symposium with Cadaver Lab

ABSTRACT #1

MSCs are Not Stem Cells

Arnold I. Caplan, PhD. Biology, Director Skeletal Research Center, Case Western Reserve University, Cleveland, OH.

Mesenchymal Stem Cells (MSCs) have the capacity to differentiate into bone, cartilage, muscle and other mesenchymal tissues in cell culture. Most, if not all MSCs, originate in vivo as perivascular cells (pericytes) and are found in every vascularized tissue of the body. When blood vessels are broken or inflamed, the pericytes come off the blood vessel and become activated MSCs. This in vivo, site- and injury-specific-MSC senses its surroundings and responds by secreting bioactive factors that are immunomodulatory and trophic establishing an immuno-protected field of tissue regeneration. Although fat, muscle and marrow-derived MSCs can be observed to be immuno-modulatory and trophic, thus the "same", their chemistries, response properties and differentiation capacities (in culture) are quite different. Importantly, PRP functions to liberate and activate MSCs so that PRP's therapeutic activities are, in part, attributed to the site-specific management of intrinsic MSCs.

The immuno-modulatory and trophic capacities of ALL human adult stem cells are likewise the same yet their differentiation programs and lineage pathways of their progeny are quite different. Considering that hematopoietic stem cells (HSCs), neurostem cells (NSCs) and the muscle (satelite) stem cells and others are all quite different in their tissue localization, they are all paracrine, have their unique niche attached to blood vessels, they are mitotic and dominant, and they are all immunomodulatory and trophic. Thus, these stem cells are all "the same." The details of MSCs and their therapeutic functionality will be stressed, but the treatise that ALL adult stem cells are alike will also be explored with the view that MSCs are not STEM CELLS in vivo compared HSCs and NSCs.

ABSTRACT #2

Biological Augmentation of ACL Reconstruction

Ramon Cugat, MD, PhD. Garcia Cugat Foundation, Barcelona, Spain.

Anterior Cruciate Ligament (ACL) injuries have a high incidence amongst athletes, which leads to a high economic impact. The purpose if the study is to assess the safety and feasibility of injecting adipose-derived regenerative cells (ADRCs) into the graft tissue during ACL reconstruction surgery in high performance athletes. The study group comprised 20 patients with complete ACL rupture. In each patient, adipose tissue was harvested from the abdomen or inner side of the thigh by a liposuction. The lipoaspirate was introduced in an automated cell processing system for preparation of ADRCs. An ACL bone tendon bone (BTB) reconstruction was performed where the graft was infiltrated with the ADRCs obtained. Clinical outcome was evaluated with MRI, VAS, IKDC, Tegner Activity, Lysholm scale and Lequesne index for grading knee pain and function. Data from a placebo control group from a study with the same surgical team, same institution and same rehabilitation protocol was used as a historical control. The MRI results assessed that the process of maturation had started within 2 months and the largest improvement occurs between the 4th and 6th month. The VAS, the Lequesne total score, the Tegner scale, the IKDC and the Lysholm Scale showed improvement in comparison to placebo control taken from our previously published study. ADRC administration to ACL BTB reconstruction was associated with a more rapid recovery from surgery relative to the control group. The implications are that the rehabilitation process may be shorter following the use of ADRCs, which may lead to a more rapid return to sport activities.

Healthy Aging Research (2017) 6:e10

Published online 24 May 2017

http://dx.doi.org/10.1097/HXR.0000000000000010

ABSTRACT #3

"Seeing is Believing" – Imaging Perspectives on Whole Spine Restoration Using Regenerative Injections

Rahul Desai, MD. RestorePDX, Beaverton, OR.

The success of spinal regenerative medicine injections hinges on accurate diagnosis and precise intervention. Identification of pain generators in the spine is particularly difficult, because many conditions mimic each other in presentation. Musculoskeletal radiology plays a key role in the proper diagnosis of spine pain by providing a delineated approach. This allows for proper identification of pain generating tissues enabling appropriate and targeted interventions. In addition, repeat imaging provides an objective measurement of regenerative medicine outcomes by visualizing tissues changes. This presentation will discuss the proper diagnosis of spine pain using musculoskeletal radiology and present the results of regenerative medicine injections in the treatment of joint, ligamentous, muscular and discogenic spine pain.

ABSTRACT #4

What is the Evidence: Specific PRP Formulation per Diagnosis

Jason L. Dragoo, MD. Stanford School of Medicine, Redwood City, CA.

There is mounting clinical evidence that standard formulations of platelet rich plasma (PRP), and related blood products, do not show a consistent clinical effect. This has lead to decreasing reimbursement for its use in the clinic and the operating room. However, randomized controlled trials do show a reproducible effect of non-neutrophil containing PRP for the treatment of osteoarthritis symptoms. This finding suggests that new formulations of PRP may achieve more efficacy than standard formulations for certain indications.

The future of PRP therapy will likely consist of indication-specific formulations of PRP whose content will vary due to the deliberate removal of certain components of PRP such as WBCs, growth factors or even platelets. Each new formulation will need to be tested first by in- vitro studies followed by human clinical trials.

Additional future strategies may also employ filtering of the plasma to increase the concentration of certain biologically active proteins to augment the anti-inflammatory or regenerative capacity of the injection. Apheresis may also be used to concentrate proteins or growth factors, or to produce specific proportions of cells within the formulation. Apheresis may also be used to enrich the PRP with peripheral-blood mesenchymal stem cells as well.

ABSTRACT #5

Individualizing Platelet-Rich Plasma Composition May Lead to Optimal Care and Treatment

Peter A. Everts, PhD. Da Vinci Clinic, Eindhoven, Netherlands.

Normal healthy tissues have the ability to spontaneously regenerate during the remodeling phase after injury, trauma, or surgery. However, if the normal healing

time of a lesion site is exceeded, a critical tissue regeneration phase may develop, leading to prolonged dysfunction and pain. An acute injury might turn to a chronic condition, like in many musculoskeletal, tendon, disorders. A disturbed tendon healing has the potential for the formation of unwanted scar tissue, leading to inferior mechanical strength, making them susceptible to re-injury.

In recent years, the injection, or implantation, of platelet-rich plasma (PRP) has been increasingly used in a variety of medical conditions, with varying outcome results, like orthopedic surgery, chronic wound care, dermatology, plastic surgery, dentistry, and sports medicine. More specifically, the efficacy of PRP treatments on injured tendons is highly debated with controversial results in vitro, animal, and clinical studies. No study has up until now demonstrated the exclusive role of PRP in enhancing tissue/tendon healing. An explicit lack of knowledge on specific roles of cells and molecules in PRP, and a large variability of PRP producing centrifuges might initiate this, as different PRP producing devices yield different PRP recipes.

In this presentation I reason that there are many PRP- and patient-related factors that influence the results of PRP treatment on injured tendons in musculoskeletal disorders. Specific emphasis will be given to the "characters" of all leukocytic cells, highlighting the granulocytic and mononuclear cell activities, and the role of macrophages, with regard to their function, and part in antimicrobial and pro-inflammatory activities. Ultimately, a variety of PRP cells and cytokines have an effect on tendon stem/progenitor cell healing. Furthermore, the erythrocyte presence in PRP will be discussed.

It is my belief that PRP should by engineered and applied based on tissue type, and disorder specific conditions, in order to create the optimum PRP milieu to significantly effect the healing mechanisms. However, only very few PRP devices are capable of producing customized treatment protocols, with a specific platelet-rich plasma composition for effective healing of, among others, musculoskeletal injuries.

ABSTRACT #6

Intraosseous Infiltrations of Synovial Derived Stem Cells for Osteoarthritis: A New Approach

Mikel Sánchez Álvarez, MD. Arthroscopic Surgery Unit, Vittoria-Gasteiz, Spain.

Osteoarthritis (OA) is a degenerative disease of the synovial joints whose main symptoms are pain, loss of motion and deformation of affected joints. The pathogenesis of osteoarthritis consists in the break of articular homeostasis, which is maintained by all structures on the joint. Recent studies show the crucial role of the subcondral bone and its communications with cartilage on osteoarthritis progress. Therefore, our group has developed a technique for severe knee osteoarthritis by means of which, the Platelet Rich Plasma (PRP) is injected directly into the subchondral bone.

Methods: These PRP intraosseous infiltrations are conducted into the femoral condyle and tibial plateau, followed by an intraarticular injection; then, two more PRP intraarticular injections are applied in a weekly basis. In order to evaluate the efficacy and safety of this technique, we conducted a Phase II clinical trial with 14 patients diagnosed with tibiofemoral knee osteoarthritis with grade III and IV based on Ahlbäck scale.

Discussion: After six months, there was a statistically significant improvement in all areas of Knee Injury and Osteoarthritis Outcome Score (KOOS), whit no adverse events. In addition to the clinical evaluation, we also analyzed Mesenchymal Stem Cells (MSC) present in synovial fluids of patients, before and after one week intraosseous infiltration. We observed a significant decrease of MSC one-week post-intraosseous injection, in both flow cytometry as cultures of colony-forming cells (CFU-F).

Conclusion: These results could indicate PRP intraosseous infiltration may stimulate the subchondral bone-cartilage unit, improving the knee biological environment and modifying pathogenesis of osteoarthritis; as a result, MSC presented in synovial fluids are modulated.

ABSTRACT #7

BMAC and Hyalofast Scaffold for Cartilage Repair: 10 Years Experience

Alberto Gobbi, MD. Oasi Bioresearch Foundation Gobbi Onlus, Milan, Italy.

Background: Chondral lesions in athletically active individuals cause considerable morbidity and treatment with existing cell based therapies can be challenging. Bone marrow has been shown as a possible source of multipotent stem cells (MSCs) with chondrogenic potential and is easy to harvest during the same surgical procedure.

Purpose: To show the clinical outcomes in a group of active patients with large full-thickness chondral defects of the knee treated in one-step surgery with MSCs derived from bone marrow and a second generation matrix over a period of time.

Methods: A systematic review of multiple medical databases was performed evaluating clinical and basic science studies to determine the effects of chondral lesion treatment. All patients underwent a HyaloFast scaffold and Bone Marrow Aspirate Concentrate surgical procedure (open or arthroscopical) and a standard post-operative rehabilitation program.

Results: Pre-operative average values in the evaluated scores (KOOS, IKDC, VAS, Tegner) were significantly improved to final follow-up.

Conclusion: Treatment of large chondral defects with multipotent stem cells showed improvement over the years and nowadays is an effective procedure that can be performed routinely in clinical practice.

ABSTRACT #8

Biologics and BMC Use in the Athlete: The Andrews Experience

Josh Hackel, MD. Andrews Orthopaedic & Sports Medicine Center, Gulf Breeze, FL.

The Andrews Institute is at the forefront of Orthobiologics and Bone Marrow Aspirate (BMA) research and application. This presentation will review prior, current and future studies that the institute is engaged in. Highlights include the treatment and successful outcomes with BMA for osteoarthritis treatment in retired NFL athletes, augmentation of ACL reconstruction with BMA and orthobiologics, quantification of fat pad stem cell harvesting, and augmentation of cartilage repair with BMA, Stem Cells.

ABSTRACT #9

Regulatory Affairs and Biologics: A Clinician's Perspective

Josh Hackel, MD. Andrews Orthopaedic & Sports Medicine Center, Gulf Breeze, FL.

Slides & video lecture online & flash drive no abstract

ABSTRACT #10

Regenerative Medicine for the Treatment of Cartilage and Bone Injuries and Disease

Johnny Huard, PhD. University of Texas Health Science Center at Houston, Houston, TX.

Introduction: The management and treatment of orthopaedic injuries has improved greatly over the last two decades with the advent of minimally invasive operative techniques and sophisticated rehabilitation, augmented by the always increasing knowledge of biomechanics and tissue engineering. Despite the progress, scientists and orthopaedic surgeons continue to struggle with the limited healing capacity of damaged structures such as articular cartilage defects and atrophic fracture non-unions. Tissue engineering and regenerative medicine are a rapidly evolving fields that focus on creating living tissue to repair, replace, or improve diseased tissue. The main goal of tissue engineering is to construct biomaterials that are capable of integrating bioactive molecules (e. g. growth factors) and/or cells. Tissues can be synthesized via both *in vitro* and *in vivo* techniques and can be made to resemble components of virtually every mammalian organ system. No matter what techniques are used or which organ system is mimicked, tissue engineering requires four critical components: (1) Production of stem cells, progenitor/precursor cells; (2) Conduction matrices or scaffolds to promote cell attachment and cell growth; (3) Induction utilizing signaling proteins, cytokines, growth factors, etc. to stimulate cellular proliferation, differentiation etc.; (4) Mechanical and biomechanical force stimulation, such as shear or strain stress, to acclimate the engineered tissue. **Stem Cells:** The "production" component involves the isolation and expansion of cellular precursors that may include single or multiple cell types that are at various levels of maturity ranging from embryonic to fully mature cells. These cells include stem cells and the various progenitor cells that they become. Stem cells are responsible for the

development and regeneration of tissues and organs. Biochemical and biomechanical signals trigger the proliferation and differentiation of stem cells during early development and during regeneration after injury or disease. Stem cells may be of post-natal or embryonic origin. Post-natal stem cells have been found in many tissues throughout the body including, skin, muscle, bone marrow, brain, liver, etc. The primary characteristics of post-natal stem cells include: (1) Their ability to differentiate into multiple lineages, their ability to self-renew, and their capacity for long term proliferation. Although post-natal stem cells reside in specialized tissues throughout the body, they retain plasticity in their ability to differentiate into multiple tissue types. For example, bone marrow derived stem cells can differentiate into skeletal muscle and muscle-derived stem cells can differentiate into osteogenic cells. Additionally, stem cells are capable of self-renewing their own stem cell populations, thereby enabling future rounds of tissue regeneration. Recently, numerous laboratories, including ours, have identified the walls of the blood vessels (endothelial cells and pericytes) as a potential niche (source) of numerous post-natal stem cells. This finding can explain why most of post-natal stem cells have been derived from well-vascularized tissue such as skeletal muscle and adipose tissue, which also highlights a potential approach to improve musculoskeletal tissue healing via the promotion of angiogenesis through exercise and neuromuscular electrical stimulation. Muscle-derived stem cells (MDSCs) obtained by the modified-preplate technique have great potential to mediate tissue repair. With their superior transplantation behavior and ability to tolerate *in vitro* manipulation, MDSCs have been shown to be useful for improving musculo-skeletal repair processes. Orthopaedic surgeons must deal with many biological challenges, such as the regeneration of articular cartilage and the inhibition of fibrosis in injured muscle. The clinical use of stem cells, particularly MDSCs, genetically manipulated to produce therapeutic growth factors or cytokines may soon become a reality. Indeed, clinical application of autologous MDSCs for urological dysfunction and cardiac injury has already been initiated.

Articular Cartilage: Perhaps one of the most challenging problems in orthopaedic surgery is the repair and regeneration of articular cartilage. In contrast to bone, articular cartilage, with its lack of blood supply and chondrogenic potential, regenerates poorly. Multiple surgical techniques, including debridement and resurfacing, subchondral drilling and microfracture, and abrasion techniques, have been employed in an attempt to coax osteochondral progenitors from the bone marrow to participate in the repair process. However, the cartilage formed after utilizing these techniques often lacks proteoglycans and the type II collagen which is responsible for making native cartilage structurally sound. Newer strategies include the use of autologous chondrocyte transplantation to the site of the cartilage defect, but this technique is complicated by the requirement of harvesting chondrocytes via an invasive arthroscopic procedure and the subsequent in vitro expansion of the cells in culture. Investigators have tried to overcome these limitations by generating chondrocytes from mesenchymal stem cells in culture. Muscle-derived cells may also be useful in the healing of articular cartilage defects. When muscle-derived cells were transplanted into full-thickness articular cartilage defects in rabbits, these cells improved the healing of the defect with an efficacy equivalent to chondrocyte transplantation, and showed better incorporation and type II collagen expression for up to 24 weeks post-transplantation. This technique is particularly appealing because muscle cells are easier to harvest and culture than chondrocytes. This technology has also been tested in an animal model of osteo-arthritis (OA) and the best stem cell regimen was obtained via genetic modification of the MDSC to express Bone Morphogenetic Protein 4 (BMP4) and an anti-angiogenic soluble receptor for Vascular Endothelial Growth Factor (VEGF), soluble fms-like tyrosine kinase-1 (s-Flt-1).

Bone: Although bone typically has an excellent potential to heal within the musculoskeletal system, non-unions or mal-unions of fractures can be quite debilitating. In sports medicine stress fractures also present a challenge due to the recovery period required, typically between 4 to 12 months or longer; though biological interventions, such as growth factor delivery, have shown promise for improving bone healing. To optimize bone healing utilizing principles of tissue engineering, one needs to be cognizant of osteo-conductive, osteo-inductive, and osteo-productive elements. For example, various scaffolds made of collagens and crystalline lattices are available for osteo-conduction. Likewise, growth factors such as BMP2 and BMP4 have been shown to be strong osteo-inductive agents. Muscle derived cells and gene therapy techniques show promise as a means to introduce osteoproductive progenitors to a fracture site. Muscle tissue has demonstrated osteogenic competence in response to osteo-inductive stimuli and muscle-derived cells have been shown to effectively deliver osteo-inductive genes to a variety of tissues. The discovery that MDSCs, which can differentiate into an osteogenic lineage and improve bone healing, suggests that skeletal muscle constitutes a vast source of osteoprogenitor cells that could be used to improve fracture healing. Indeed we have shown that MDSCs genetically engineered to expresse either BMP2 or BMP4 can improve bone healing and furthermore demonstrate that angiogenesis play an important role in the bone repair process.

Future Directions: Gene therapy applications must be refined to overcome many obstacles before achieving the status of current established therapeutic techniques. The successful delivery of therapeutic genes to the human joint has been documented, and many animal studies have indicated a benefit to the delivery of different genes to tissues throughout the musculo-skeletal system. One of the main obstacles impeding the application of gene therapy to humans has to do with the availability of appropriate vectors to carry and deliver the required genes; however, great progress is being made in the development of new vectors. We believe that the combination of gene therapy techniques, tissue engineering principles, and the use of post-natal stem cells, including MDSCs, could result in establishing new and effective therapies for improving the healing of tissues with low regenerative capacities (e. g. articular cartilage); however, numerous basic science and pre-clinical studies need to be performed before these techniques can be preformed with the level of efficiency and safety required for clinical orthopaedic applications. Since we have observed that after implanting MDSCs within an injured area, that the repair process is mediated by chemo-attracted host cells, a major focus of our laboratory involves the elucidation of the origin of these host cells and the development of methods to further promote cross-talk between the donor and host cells. ***In this presentation,*** we will review the current knowledge concerning the use of tissue engineering applications based on MDSCs to improve the healing of bone and articular cartilage.

ABSTRACT #11

Lumbar Intradiscal Platelet Rich Plasma Injections for Discogenic Low Back Pain

Gregory E. Lutz, MD. Hospital for Special Surgery, New York, NY.

The pathophysiology of intervertebral disc degeneration (IDD) remains incompletely understood. Accordingly, current treatments for IDD are primarily focused on alleviating symptoms without addressing their underlying causes. PRP is a biologically active injectate that lends itself as an attractive treatment for symptomatic annular fissures. In this study, adults with chronic ($\geq$ 6 mo) moderate to severe lumbar discogenic pain unresponsive to conservative treatment were randomized to receive intradiscal PRP or additional contrast agent following provocative discography. Data on pain, physical function, and participant satisfaction were collected at 1 week, 4 weeks, 8 weeks, 6 months, and 1 year. Participants in the control group who did not improve at eight weeks were offered the option to receive PRP and were subsequently followed. Forty-seven participants (29 received treatment, 18 received control) were analyzed by an independent observer with a 92% follow-up rate. Over eight weeks, there were statistically significant improvements in participants who received PRP with regards to pain ($P = 0.02$), function ($P = 0.03$), and patient satisfaction ($P = 0.01$) when compared to controls. Those who received PRP maintained this level of improvement through at least two years of follow-up. These significant improvements were not observed among those in the control group. No adverse events of disc space infection, neurologic injury, or progressive herniation were reported following the injection of PRP. While these results are encouraging, further studies are needed in order to define the subset of patients most likely to respond to biologic intradiscal treatment and the ideal cellular characteristics of the intradiscal PRP injectate.

ABSTRACT #12

Understanding the Anatomy of Subchondral Bone & Cartilage

Henning Madry, MD. Institute for Experimental Orthopaedics and Department of Orthopaedic Surgery, Saarland University Medical Center, Homburg, Germany.

The subchondral bone and articular cartilage form the osteochondral unit. From an anatomical standpoint, the subchondral bone is the bony lamella that lies below the calcified zone of the articular cartilage, separated by the cement line. The subchondral bone consists of the subchondral bone plate and the

subarticular spongiosa. It is separated by the cement line from the calcified zone of the articular cartilage. A variable anatomy is characteristic for the subchondral region, reflected in differences in thickness, density, and composition of the subchondral bone plate, contour of the tidemark and cement line, and the number and types of channels penetrating into the calcified cartilage. Trabeculae arising from the subchondral bone plate form a spongious network, the subarticular spongiosa. The term osteochondral unit reflects the close interaction between these two tissues. A profound understanding of the basic anatomic aspects of this particular site, together with the pathophysiology of diseases affecting the subchondral bone is the key to develop targeted and effective therapeutic strategies for osteochondral repair. This talk aims at providing insights into the anatomy, morphology, and pathology of the subchondral bone and the articular cartilage. Individual diseases affecting the subchondral bone, such as traumatic osteochondral defects, osteochondritis dissecans, osteonecrosis, and osteoarthritis are discussed. Clinical relevant knowledge of the basic science of the subchondral region, together with additional investigations in animal models and patients is provided, together with improved strategies for articular cartilage defect repair in the context of the subchondral bone.

ABSTRACT #13

An Evidence-Informed Approach to Rehabilitation Following Orthobiologic Procedures

Kenneth R. Mautner, MD. Emory Sports Medicine Center, Atlanta, GA.

There are various rehabilitation protocols are in use following biologic treatments such as platelet rich plasma (PRP) and stem cells for soft tissue and cartilage injuries. Critical review of current literature found that protocols share recommendations to avoid use of NSAIDs initially after biologics, favor early mobilization, and include a progressive exercise program over the course of 12 weeks before unrestricted activity. Along with varying opinions regarding the use of cyrotherapy, weight baring restrictions, timing of mobilizations and eccentrics. This lecture will review trends in rehabilitation following orthobiologic procedures and present evidenced based recommendations based on physiological mechanisms and current outcome data.

ABSTRACT #14

Mechanical Processing of Lipoaspirate for MSC Acquisition

Laurence McClish, MD and Andrew C. Wesely, MD. Sierra Stem Cell Institute, Reno, NV.

Lipoaspirate has been obtained on 10 patients to date using tumescent liposuction. The cells have been processed by mechanical means using a simple and reproducible process. A small portion of the stem cells acquired on all patients has been submitted for flow cytometry analysis at the University of Nevada, Reno. To date, the preponderance of the cellular analysis has shown CD 34 positive, and also CD 105 positive stem cells. There have been some CD 90 positive stem cells also. The average viable stem cell count on 110-120 mL of fat processed is approximately 2,000,000 or greater. The total nucleated cell count is, of course, higher. The viability on all stem cells has been greater than 90%.

The stem cells are subsequently mixed with resting PRP and also activated PRP for injection into the targeted joint. To date osteoarthritis is the main treatment diagnosis. Most joints treated have been knees, but treatment also has included shoulders. In general, early results have been promising, and equal enzyme results.

At the point of this writing, many more cases are scheduled in the upcoming few months. It is anticipated that we will have more than 25 patients in the study by March 2016. As has been done so far, each patient will continue to have initial pain scores, complete individual stem cell analysis, and follow-up pain scores.

We also plan on submitting our study to a major stem cell journal.

Dr. Wesely and myself believe that this is a major advance in autologous point-of-care stem cell therapy, mainly because our simple mechanical process for the acquisition of stem cells from adipose tissue equals the enzymatic process regarding MSC yield and viability. It is also a simple, low-cost method which should not pose any regulatory concerns and is relatively easy to implement with reproducible results. This is also the first time a fresh tissue sample of autologous adipose-derived MSC's has been characterized prior to culturing. It is a true representation of what is actually being delivered to the patient at the point of care.

ABSTRACT #15

Platelet Rich Plasma and Adipose Stem Cells: Application Specific Combination of Autologous Biologics

Randy B. Miller, MD. Cosmetic & Reconstructive Surgery, Coconut Grove, FL.

Purpose: To provide scientific rationale, literature review, application specific techniques, clinical examples, and outcome analysis of an autologous treatment modality that utilizes a combination of platelet rich plasma and adipose tissue containing mesenchymal stem cells.

Background: Platelet rich plasma (PRP) and adipose derived mesenchymal stem cells (AMSC) have been used alone and in combination to achieve regeneration of injured tissues. Growth factors, angiogenic factors, cytokines, lymphokines and plasma proteins are highly concentrated in PRP. The anti-inflammatory effects of PRP have been shown to greatly exceed the pro-inflammatory effects, particularly with the minimization of erythrocytes, leukocytes and granulocytes. The antimicrobial and hemostatic properties of PRP are well established. There are no known deleterious side effects associated with PRP. In vitro, PRP and platelet lysate have been shown to increase AMSC viability, proliferation, differentiation and minimize apoptosis. The industry standard for preparation of PRP from whole blood has yet to be defined and varies widely, leading to inconsistent product, outcomes and extensive controversy. While the combination of PRP, AMSC and adipose tissue is supported scientifically and clinically, the technique for combination and optimal ratios for specific applications have not been elucidated.

Methods: A prospective randomized double-blind trial was performed in 64 cases of moderate wrist arthritis which included 26 acute cases and 38 chronic cases. All patients were treated with PRP alone or in combination with minimally manipulated adipose tissue containing AMSC. The preparation of PRP and AMSC were standardized to minimize variability. Patients were followed from 10 days to 24 months and outcomes were measured in comparison with the pre-treatment Mayo Wrist Score and the Disabilities of the Arm Shoulder and Hand (DASH) Score.

Summary of Results: The results of treatment with PRP alone or in combination AMSC in cases of moderate acute wrist arthritis were similar with no significant variation. The results of treatment with PRP alone or in combination AMSC in cases of moderate chronic wrist arthritis varied significantly with the combination of PRP and AMSC being the most efficacious.

Discussion and Conclusion: Platelet rich plasma (PRP) is a safe and efficacious adjunct for treatments utilizing AMSC and adipose transfer. Standardization of PRP and adipose preparation is an important first step. With a uniform preparation technique, it will be possible to determine optimal compositions, concentrations, volumes, and ratios for specific clinical applications. Only then will we be able to assess efficacy and generate evidence based reproducibility.

ABSTRACT #16

Biologic Orthopedics: From PRP to Precision Medicine

Allan K. Mishra, MD. Menlo Medical Clinic: Stanford Hospital, Menlo Park, CA.

In 2016, Biologic Orthopedics is exploding in popularity. This popularity is being driven by the more than 125 million Americans affected by musculoskeletal conditions. Collectively, the cost to treat these injuries and disorders exceeds $200 billion dollars annually and accounts for 16% of all healthcare costs. The number of PRP references now is over 8400 total on PubMed and there are more than 200 on-going PRP clinical trials. The use of cell based products and growth factors has been identified as a research priority by several organizations including the AAOS and ORS. A new organization dedicated to the biologics recently started and now has over 5500 members. (The Biologic Orthopedic Society / BiologicOrtho.com) Importantly, new legislation has been introduced in Congress to accelerate the approval of regenerative medicine. The ReGrow Act, also known as HR 4762 has been proposed to amend the FDA with regard to cellular therapies.

Platelet-rich plasma is complex and needs to be more precise. More than 20 different versions exist with several classification systems. We need to better understand PRP as a biologic therapy. PRP represents a form of personalized

medicine but is not yet precision medicine. In depth analysis of genomic data and the exposome will enhance our comprehension of how PRP works. Finally, a novel formulation of PRP will be introduced during the lecture that differentially fractionates whole blood and white blood cells.

ABSTRACT #17

Disc and Epidural Biologics Outcome Data: Efficacy and Sustainability

Annu Navani, MD. Comprehensive Spine and Sports Center, Campbell, CA.

Introduction: Low back pain affects a large portion of the population causing a major social and economic impact. Current interventional treatments remain inadequate and transient targeting the symptoms without addressing the underlying cause. Discogenic low back pain is a serious medical and social problem, and accounts for 26-42% of the patients with chronic low back pain. Biologics including Platelet-rich Plasma (PRP) and Mesenchymal stem cells are gaining a lot of attention with regards to their application to treat spine pain. In vitro animal studies have demonstrated the intradiscal biologics injection to decrease inflammation and pain and restore disc height in some cases.

Objective: The objective is to study the safety and efficacy of epidural platelet rich plasma injection and intradiscal biologic injections. In order to study efficacy and sustainability we have followed the patients for 18-24 months and are reporting the data at the meeting. We have also compared outcomes between epidural PRP and conventional epidural steroid injection (ESI) patients over a period of 6 months.

Methods: All spine injections were completed under strict asepsis and fluoroscopy guidance in an Operating room environment. The epidural injections were completed via interlaminar or transforaminal approach. The patients with chronic discogenic low back and leg pain who tried and failed conservative treatments were administered a single injection of intradiscal PRP or BMA into the nucleus pulposus after careful study of the disc anatomy. The patients were followed until a period of 18 months. The primary outcome was efficacy and safety and the secondary outcomes included change in function, medication use, hospitalization and spine surgery.

Results: The Epidural PRP patients sustained benefits for 18 months although the minimum VAS achieved was not maintained in all patients at 18 months. Epidural PRP patients demonstrated slightly better and longer relief than ESI. In the intradiscal biologic group (PRP and BMA) the pain relief of $> 50\%$ maintained in a majority of patients at the 18 month mark. The 6- month data was also reproducible in a separate new cohort. There were no complications in any patient. None of the patients presented to the hospital or received surgery after this treatment.

Conclusions: While our preliminary results have been promising, well-designed randomized controlled studies are warranted in order to understand the full breath of the efficacy, risks, and complications from the use of biologics in the spine.

ABSTRACT #18

Understanding Regulations in Regenerative Medicine: What You Need to Know

Karl Nobert. Food and Drug Regulatory Attorney, ReCellerate, Inc., Middlleburg, VA.

Stem cells intended for therapeutic purposes in humans are regulated as biologics under FDA's April 2006 regulations governing the use of human cells, tissues, and cellular and tissue-based products ("HCT/Ps"). These regulations define HCT/Ps as "articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient." Among other things the regulations include provisions governing their use in clinical practice, requirements intended to control the spread of communicable disease and the marketing of such therapies to patients. This presentation will provide attendees with an overview of FDA's stem cell regulations, an understanding of the criteria that FDA uses to determine a product's regulatory status for marketing and sale; the rules governing the use of stem cells and other regenerative medicine products like platelet rich plasma in in the clinical; and practical strategies for physicians to mitigate the risk of FDA and state enforcement action.

ABSTRACT #19

Bone Marrow Concentrate to Treat Osteoarthrits: Current Evidence Base and Future Directions

Shane A. Shapiro, MD. Mayo Clinic College of Medicine Department of Orthopedic Surgery, Jacksonville, FL.

No abstract available, slides and lectures available online & on flash drive

ABSTRACT #20

Allogenic Marrow-isolated Adult Multi-lineage Inducible Cell Therapy for Post Malignant Bone Healing

H. Thomas Temple, MD. Nova Southeastern University, Fort Lauderdale, FL.

Bone marrow is a rich source of mesenchymal stem cells. Autologous recovery of these cells, without cell expansion, results in inadequate numbers of poorly characterized cells. Allogeneic recovery on the other hand can produce large numbers of cells that can be well-characterized. Combined with robust bone scaffolds, these cells participate in musculoskeletal tissue repair and regeneration.

An ideal cell product is one that strictly adheres to FDA guidelines related to 361 products, specifically requiring that the product is: minimally manipulated, is used in a homologous fashion, is not combined with another article and is not administered systemically. If a cell or tissue product does not meet these standards, it is regulated under different standards.

Marrow derived mesenchymal osteogenic precursor and MIAMI (marrow isolated adult multilineage inducible) cells placed on a robust bone matrix has been observed clinically to form new bone in a rapid fashion in conditions such as allograft-host non-unions and radiation osteonecrosis. This cell product is superior to autogeneic bone and marrow aspiration for these purposes.

ABSTRACT #21

Poster—Intra-articular Bone Marrow Concentrate (BMC) Injection Protocol: Short Term Efficacy and Safety for Osteoarthritis

Steven Sampson, DO*, Jay Smith, MD†, Hunter Vincent, DO*, Danielle Aufiero, MD*, Mona Zall, DO‡, and Angie Botto-van-Bemden, PhD*. *Orthohealing Center, Los Angeles, CA; †Mayo Clinic, Rochester, MN; ‡University of California, Los Angeles, CA.

Objective: To evaluate the short-term safety and efficacy of a single intra-articular injection of autologous, non-culture expanded bone marrow concentrate (BMC), followed by an 8 week follow up injection of platelet rich plasma (PRP) in moderate to severe osteoarthritis (OA).

Design: Single center, retrospective clinical trial of 125 patients treated with a single intra-articular injection of BMC, followed by an 8 week follow up injection of PRP for symptomatic moderate to severe osteoarthritis affecting the ankle (n = 6), bilateral knees (n = 27), cervical spine (n = 4), hip (n = 14), unilateral knee (n = 46), shoulder (n = 18), or other joint (n = 9) with a median follow-up of 148 days (25th percentile = 89 d, 75th percentile 222 d; minimum follow up for inclusion was 56 d). Patient reported outcomes (PROs) included Visual Analog Pain Scale and a patient satisfaction questionnaire.

Methods: Bone marrow was aspirated utilizing a standardized technique from the PSIS(s) of each patient, and concentrated using double-spin centrifugation (10 min at 2800 RPMs, and 6 min at 3400 RPMs). Patients received a single intraarticular injection of bone marrow concentrate into the affect joint region (s) using ultrasound or fluoroscopic guidance, without additives. Eight weeks following BMC injection, patients received and additional injection of autologous PRP (concentrated using the same double spin centrifugation technique) to the affected joint(s). Patients completed a pre and post procedure VAS score, as well as a patient satisfaction survey.

Protocol:

Step 1: Bone Marrow Aspiration

Step 2: Bone Marrow Concentration:

- Approximately 60 cc bone marrow spun for 10 minutes at 2800 RPMs
- Platelet Poor Plasma (PPP) and Buffy Coat layers removed and centrifuged again for 6 minutes at 3400 RPMs
- PPP removed and remaining cellular mixture is the finalized BMC

Step 3: Intra-articular BMC Injection
- 60 cc of bone marrow aspirate makes approximately 6 cc of BMC
- Volume injected was patient and joint specific

Step 4: PRP Booster Injection
- Patients returned to clinic approximately 8 weeks post-BMC injection
- Whole venous blood was drawn and PRP was concentrated using the same double spin centrifugation process as BMC
- Cell cytometry was performed on PRP samples to insure standardization under the PLRA classification

Results:

In summary, no adverse effects were reported during the follow-up period, median pre-injection absolute pain scores were 7.0 (range 2- 10, 25th percentile = 5.0, 75th percentile = 9.0), and median post-injection absolute pain scores were 2.0 (range 0- 10, 25thpercentile = 1.0, 75th percentile = 3.0). Patients reported a median pain reduction of – 5.0 (range – 9.0 to + 6.0, 25th percentile = – 7.0, 75th percentile = – 3.0) and an average pain reduction of 71.4% compared to pre-treatment values ($P < 0.0001$). Median patient satisfaction was 9.0 out of 10, 91.7% (77/84) of patients indicated that they would repeat the procedure, and 94% (79/84) indicated that they would recommend the procedure to a friend.

ABSTRACT #22

Poster—Osteoarthritis: Can it be Reversed A Novel, Minimally Invasive Treatment that Employs Bone Marrow for Relief of Advanced Gonarthrosis

Bülent Kılıç*, Feyza Ünlü Özkan†, Murat Demiroğlu‡, Ismail Turkmen§, Yavuz Saglam‖, and Korhan Özkan¶. *Istanbul Gelişim University, Department of Health Sciences, Orthopaedic surgeon, Turkey; †Fatih Sultan Mehmet Education and Research Hospital, Department of Physical Therapy and Rehabilitation, Turkey; ‡Istanbul Medeniyet University, Goztepe Training and Research Hospital, Department of Orthopaedics and Traumatology; §Beykoz State Hospital, Department of Orthopaedics, Turkey; ‖Biruni University faculty of Medicine, Department of Orthopaedics, Turkey; ¶Istanbul Medeniyet University, Faculty of Medicine, Department of Orthopaedics and Traumatology, Turkey.

Introduction: Osteoarthritis of the knee is leading cause of disability in the elderly. Although there are pharmacological and nonpharmacological treatment methods, these are generally insufficient to alleviate pain and the disability in advanced cases. Although total knee arthroplasty improves quality of life and provides a generally high level of patient satisfaction for treatment of advanced gonarthrosis, it is not always without complications.

Material and Methods: Herein we present a new technique consisting of patellofemoral joint irrigation, simple osteophytectomy if needed, lateral patellar retinaculum release, subchondral drilling of the proximal tibia, percutaneous medial collateral ligament release, intra-articular bone marrow injection, and the results of this treatment applied under local anesthesia in 15 knees of 10 patients.

Results: The mean VAS was 8.20 ± 0.68 prior to treatment and 3.33 ± 0.72 after treatment; the values were 18.67 ± 3.34 and 4.10 ± 3.15 for lequesne measurements, 7.80 ± 0.77 and 1.07 ± 0.96 for pain, 5.07 ± 2.28 and 1.80 ± 1.42 for walking, and 5.80 ± 0.92 and 1.23 ± 0.92 for daily living activities, respectively. All decreases were statistically significant ($P = 0.001$ for $P < 0.01$ in all cases).

Conclusion: Biological treatment solutions to gonarthrosis without using foreign materials could decrease the need for prosthetic surgery and its related complications, as well as the need for further attempts at revision. We propose this minimally invasive technique as a pioneer in the treatment of OA.

ABSTRACT #23

Poster—Does Patient Variability and Platelet-rich Plasma Composition Impact Treatment Outcome

Leung JP and McKenzie D. University of British Columbia, Division of Sports Medicine and LightIntegra Technology Inc., Vancouver, Canada.

Introduction: Platelet based therapies have shown a wide range of efficacy in clinical studies. Failures in platelet-based therapies can be related to donor/patient traits and platelet product characteristics. Platelet-rich plasma (PRP) generation systems primarily focus on recovering and concentrating platelets over baseline values, but do not offer detailed information on sample properties. Patient to patient variability is exhibited by the wide range in baseline cell counts and also cellular properties, such as size and microparticle (MP) content. Microparticles are an intrinsic part of blood products and have active roles in inflammation and hemostasis. The role of MPs or PRP composition on treatment success has not been assessed. PRP samples were reviewed to evaluate heterogeneity.

Methods: Whole blood anticoagulated in acid citrate dextrose were processed (150 × g for 12 min) to generate PRP. Samples were analyzed by a hematology analyzer, platelet activation assay (CD62), and MP/platelet content by dynamic light scattering and flow cytometry. Platelet properties (N = 13) of whole blood (WB) and the associated PRP were compared. The differences in PRP collection from multiple collections from the same subject (N = 6) at different time points were evaluated.

Results: Mean platelet volume (MPV) decreased by 0 to 20% and there was a moderate correlation in platelet count (r = 0.46) between WB and collected PRP. Platelet recovery was related to MPV (r = 0.53). PRP collections had differences in platelet count, platelet activation and MP content up to 40, 38 and 58% respectively.

Conclusions: Large variations in PRP collected from the same and different patients suggest that pre-screening patient PRP can be used to standardize and optimize PRP preparations or potentially effect treatment decisions. The effect of PRP heterogeneity is being evaluated in the PRiSM pilot study. The goal of this study is to ascertain if PRP composition relates to patient (N = 40) outcome (pain scores).

ABSTRACT #24

Poster—Treatment of Medial and Lateral Elbow Tendinosis with an Injectable Amniotic Membrane Allograft - A Retrospective Case Series

Danielle Aufiero, Steven Sampson, Kentaro Onishi, and Angie Botto-van Bemden. Orthohealing Center, Los Angeles, CA.

Background: Epicondylitis is the second most frequently encountered head and upper limb musculoskeletal diagnosis in primary care clinics, with an incidence rate as high as 7/1000 patients per year. Chronic or recalcitrant epicondylitis – more appropriately termed epicondylosis or elbow tendinosis – is not uncommon and represents a notable set of pathologies which account for lost recreation time, decreased quality of life, and workers compensation claims. A novel non-operative option has recently become available in the form of micronized dehydrated human amniotic/chorionic membrane (mdHACM) allograft.

Hypothesis: mdHACM allograft is known to be rich in anti-inflammatory cytokines and tissue inhibitors of metalloproteinase and IL-10. It also contains an abundance of growth factors and cytokines. *In vivo* and *in vitro* studies have shown reduction in scar tissue. We hypothesize that mdHACM allograft will be a viable treatment option in patients with epicondylosis.

Study deisgn: Retrospective case series.

Level of evidence: IV.

Methods: Chart were retrospectively reviewed for 10 patients who received mdHACM allograft injections for treatment of medial or lateral epicondylosis.

Results: There were significant decreases in pain scores and improvemtn in epicondylosis when compared to a baseline.

Conclusion: mdHACM allograft may be a safe and viable treatment option in patients with elbow tendinosis.

ABSTRACT #25

Poster—Roles for Mesenchymal Stem Cells as Medicinal Signaling Cells

Rodrigo A. Samoza, Diego Correa, and Arnold I. Caplan. Case Western Reserve University, Cleveland, OH.

Understanding the in vivo identity and function of mesenchymal stem cells (MSCs) is vital to fully exploiting their therapeutic potential. New data are emerging that demonstrate previously undescribed roles of MSCs in vivo. Understanding the behavior of MSCs in vivo is crucial as recent results suggest these additional roles enable MSCs to function as medicinal signaling cells. This medicinal signaling activity is in addition to the contribution of MSCs to the maintenance of the stem cell niche and homeostasis. There is increasing

evidence that not all cells described as MSCs share the same properties. Most MSCs reside in a perivascular location and have some functionalities in common with those of the pericytes and adventitial cells located around the microvasculature and larger vessels, respectively. Here we focus on the characteristics of MSCs that have been demonstrated to be similar to those of pericytes located around the microvasculature, defined as perivascular MSCs (pMSCs). Although we focus here on pMSCs, it is important to bear in mind that pericytes are found in many types of blood vessels, and that not all pericytes are thought to be MSCs.

ABSTRACT #26

***Poster*—Treatment of a Full Thickness Tear of Supraspinatus with PDRN (Polydeoxyribonucleotide): A Case Report**

Shi-Uk Lee, MD. Seoul National University Hospital, Seoul, South Korea.

Setting: Outpatient clinic of a university affiliated hospital.

Patient: 71 years old female patient.

Case Description: This patient has suffered from right shoulder pain for 4 months. On MRI, she had full thickness tear of supraspinatus (SS) tendon. The intensity of her pain was 7 out of 10 in visual analogue scale (VAS). She could not elevate her shoulder above 90 degrees. She complained of pain at night waking her up. She was recommended to get surgery which she did not want.

On physical examination, range of motion was full with positive Neer test and Hawkins Kennedy test. On ultrasonography (USG), full thickness tear of SS tendon was noted.

As she did not want to go on surgery, treatment with PDRN was considered as an option for conservative treatment. Under USG, 3 mL of PDRN (5.625 mg) was injected around SS tendon. Injected sites were footprint, articular side of SS and subdeltoid subacromial bursa. Total 3 injections were administered with a week interval, i. e. 3 times, 1/week.

Results: The pain on VAS decreased to 2 out of 10. She did not feel any pain except extreme shoulder elevation. On USG after 1 month of her last injection, full thickness tear of SS was still noted but seemed to be filled with fibrous tissue rather than empty.

Discussion: PDRN (polydeoxyribonucleotide), an adenosine receptor A2A agonist has been reported to be effective on wound healing such as diabetic foot ulcers. Prolotherapy with dextrose for refractory rotator cuff disease has been reported to be effective over control group. This case was an example case of treating full thickness SS tear with prolotherapeutic approach with an A2A agonist which is effective for wound healing.

Conclusion: Injection of PDRN as a prolotherapeutic agent to the SS may be a useful option when either the condition of the patient is not suitable for surgery or the patient does not want to get surgery.