ORIGINAL ARTICLE

Bone Marrow Derived-Mesenchymal Stromal Cells and Platelet-Rich Plasma on a Collagen Matrix to Improve Fascial Repair

Jeremy J. Heffner, MD*, Jonathan W. Holmes, MD*, Jonathan P. Ferrari, MD*, Johanna Krortiris-Litowitz, PhD†, Hazel Marie, PhD‡, Diana L. Fagan, PhD†, John C. Perko, BS†, Heath A. Dorion, MD*

*Department of General Surgery, St. Elizabeth Health Center / Northeastern Ohio University College of Medicine, Youngstown, OH
Departments of †Biological Sciences and ‡Mechanical and Industrial Engineering, Youngstown State University, Youngstown, OH

Corresponding Author & Requests for Reprints: Jeremy Heffner, MD
Department of Surgical Education, St. Elizabeth Health Center
1044 Belmont Ave., Youngstown, OH 44501
Phone: (330) 480-3124; Fax: (330) 480-3640; Email: bigheff11@yahoo.com

Conflicts of Interest and Source of Funding: Funding provided by the American Hernia Society/Lifestyle Resident Research Award 2010. There are no other disclosures.

Running Head: Cell-based Therapy for Hernia Prevention
Bone Marrow Derived-Mesenchymal Stromal Cells and Platelet-Rich Plasma on a Collagen Matrix to Improve Fascial Repair
(Mini-Abstract)

Primary repair of a midline laparotomy with a CollaTape overlay, platelet-rich plasma, and mesenchymal stromal cells in a Lewis rat model demonstrated a statistically significant increase in tensile strength, total energy absorption, neovascularization and collagen abundance at both four and eight weeks. Application of this therapy may reduce incisional herniation.
Bone Marrow Derived-Mesenchymal Stromal Cells and Platelet-Rich Plasma on a Collagen Matrix to Improve Fascial Repair

**Objective:** To demonstrate improved healing of a midline laparotomy after application of mesenchymal stromal cells and platelet-rich plasma on a collagen matrix. To introduce a potential cellular-based therapy for the prevention of ventral hernia formation.

**Background:** Up to 10% of laparotomies are complicated by postoperative incisional hernias. Despite continuous improvements in surgical technique and technology, hernia rates have remained constant. Cell-based therapies focused on augmentation of the body’s natural healing properties could reduce initial hernia formation.

**Methods:** Midline laparotomies were performed on 42 Lewis rats. Three groups were studied: (1) primary repair only, (2) primary repair with CollaTape™ (CoTa) overlay and platelet-rich plasma (PRP), and (3) primary repair with CoTa overlay and PRP and bone marrow derived-mesenchymal stromal cells (BM-MSCs). Abdominal wall fascia was recovered at four and eight weeks in each group. Biomechanical testing and histological evaluation was performed.

**Results:** At four weeks there was a two-fold increase in tensile strength between groups 1 and 2 and a four-fold increase between groups 1 and 3 (p<0.001). Group 3 had a 320% increase in total energy absorption at four weeks compared to group 1 and a 142% increase at eight weeks (p<0.001). Neovascularization and collagen abundance were significantly increased in group 3 at both time points.

**Conclusion:** The addition of BM-MSCs, PRP and CoTa led to a marked improvement in abdominal wall strength and energy absorption. Histologic evaluation confirmed increased vascularity and collagen abundance consistent with the biomechanical findings. Application of this therapy may ultimately reduce postoperative hernia formation.
Mesenchymal Stromal Cells and Platelet-Rich Plasma on a Collagen Matrix to Improve Fascial Repair

INTRODUCTION

Incisional hernias occur following approximately 10% of laparotomies.\textsuperscript{1-5} The complications of these hernias range from cosmetic disfigurement to small bowel strangulation and lead to numerous hospitalizations. More problematic is the 30-60% recurrence after hernia repair.\textsuperscript{6-7} Despite extensive research in repair strategies, techniques, and technologies, recurrent herniation continues to be a major burden on patients and the healthcare system.\textsuperscript{8} Preventative strategies would reduce the risk of herniation after fascial incision before the cycle of herniation, repair, and recurrence begins, thereby reducing morbidity and healthcare costs. Multiple techniques for fascial closure after laparotomy have been studied, but the rate of hernia formation after laparotomy remains unreasonably high.\textsuperscript{9-10} With an improved understanding of wound healing and recent progress in cell therapies, the time has come to apply this knowledge to fascial repair to effectively eliminate the vicious cycle of incisional herniation.

Optimization of the wound healing process is a key component to hernia prevention. Under the current concept of wound healing, the process transitions through three phases – inflammation, proliferation, and eventual maturation. In a sterile hemostatic surgical wound, it would be ideal to minimize the inflammatory phase and expedite the transition to the proliferative phase. Early activation and optimization of the proliferative phase should improve fascial strength and integrity resulting in decreased hernia rates in standard surgical incisions undergoing primary repair.

Current biologic technologies including Platelet-rich plasma (PRP), collagen, and bone marrow derived-mesenchymal stromal cells (BM-MSCs) have shown particular advantages in wound healing and tissue regeneration. PRP contains physiologic ratios of a variety of growth factors, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and transforming growth factor beta (TGF-β), which are found in abundance during the proliferative phase of wound healing and promote tissue regeneration.\textsuperscript{11-13} Studies have proven that PRP is beneficial in promoting healing of cutaneous wounds.\textsuperscript{14} PRP has also been shown to increase fibroblast and MSC proliferation.\textsuperscript{15,16} Collagen serves as a scaffold for cell migration and provides strength to the forming scar. These properties of collagen have been utilized for the growth of neuronal and vascular tissues and the repair of bony defects.\textsuperscript{17-20} This evidence supports PRP and collagen as a foundation for improved tissue regeneration.

First discovered by AJ Friedenstein in 1976 due to their adherence to plastic, BM-MSCs make up 0.01-0.001% of nucleated cells in the bone marrow.\textsuperscript{21,22} A large body of research exists examining the regenerative capabilities of BM-MSCs in a variety of tissues, including bone, cardiac, neuronal, connective, hepatic, endothelial and cutaneous. Badiavas et al. have shown that BM-MSCs migrate to the wound bed during normal cutaneous wound healing.\textsuperscript{23} His research demonstrated BM-MSC differentiation and incorporation into normal skin tissue. Falanga et al. have shown an increase in wound closure rates in both acute and chronic cutaneous
wounds using BM-MSCs. These known characteristics of BM-MSCs and their associated wound healing properties hold promise for their use in improved fascial repair.

Our previous work using a rabbit model suggested an advantage in using a combination of BM-MSCs and PRP on a type I collagen matrix to improve fascial strength and total energy absorption at four and eight weeks after a midline laparotomy. The goal of the current study was to definitively demonstrate that BM-MSCs, together with PRP, on a collagen scaffold dramatically improve tensile strength and total energy absorption after a primary fascial repair.

MATERIALS AND METHODS

Forty-two adult male Lewis rats weighing 250-300 grams were obtained from Charles River Laboratories International, Inc., in Wilmington, Massachusetts. These were housed in the animal care facility at Youngstown State University and acclimated for one week prior to experimental use. Standard rat chow and water was provided ad libitum. The experimental protocol for this study was approved by the Institutional Animal Care and Use Committee at Youngstown State University.

Study Design

Age-matched rats were separated into three equal groups of fourteen rats per group divided into two equal subgroups per group with 7 rats per subgroup (Table 1). Group 1 (the control) only underwent primary repair of a midline fascial incision. Group 2 underwent the same repair with the addition of a CollaTape™ (CoTa) onlay implant and platelet-rich plasma (PRP). CoTa and PRP were considered the vehicle and were tested for their combined contributions to fascial healing. Group 3 was treated the same as group 2 with the addition of BM-MSCs to the vehicle. These groups were subdivided and analyzed at four weeks (subgroup a) and eight weeks (subgroup b) postoperatively. All of the rats were housed within each group.

Collagen, PRP, and MSC Preparations

Collagen

CollaTape™ type I bovine collagen was obtained from Zimmer Dental in Mississauga, Ontario, Canada. According to the manufacturer, this collagen scaffold is fabricated using collagen obtained from bovine deep flexor (Achilles) tendons. CoTa is a sterile, pyrogen-free, porous, biodegradable biomaterial available in 2.5-cm x 7.6-cm strips. Under sterile conditions, it was cut into 1-cm x 6-cm strips for application.

Platelet-Rich Plasma

The protocol for acquisition of platelet-rich plasma was modified from the protocol by Maekawa et al. Blood (5-10 ml total per rat) was collected using a 21-gauge needle and a 10-mL syringe containing 1/10 volume of the anticoagulant citrate dextrose (ACD). The blood was centrifuged for 10 minutes at 200 x g at room temperature. The plasma layer was aspirated and centrifuged
at 700 x g for 10 minutes. The platelet-poor upper layer from this second centrifugation was aspirated and stored at -20 °C. One milliliter of this platelet-poor layer was kept with the platelet pellet which was then resuspended adding 5% (5 μL) DMSO. This preparation was then placed in a cryovial, slowly frozen to -80 °C, and stored in liquid nitrogen. Prior to application, the platelet-poor plasma which was stored at -20 °C was thawed to 37 °C. One milliliter of this plasma was placed in a separate centrifuge tube. The platelets stored in liquid nitrogen were then thawed until the ice pellet could be dislodged into the reserved 1 mL of warm plasma. This was then mixed to thaw rapidly. This mixture was centrifuged at 700 x g for 10 min at 4 °C. The plasma portion was aspirated and discarded. The platelet pellet was then resuspended in the remaining plasma. This platelet rich-plasma was then used in the experiments.

**Bone Marrow-derived Mesenchymal Stromal Cells**

The acquisition and processing of BM-MSCs was based on previous literature.²⁸,²⁹ Bone marrow was recovered from the femurs and tibias of syngeneic Lewis rats using a 21-gauge needle. The bone marrow was flushed with 30 mL of minimum essential medium (MEM) alpha media. The clumps were allowed to settle for 5 minutes. The supernatant, excluding the bottom 0.5 mL of solution containing the clumps, was collected. The supernatant containing the MSCs was centrifuged at 400 x g for 10 minutes at room temperature. The resultant pellet was removed and resuspended in 10 mL of complete media (MEM alpha media containing 20% fetal calf serum (FCS), 2 mM of L-glutamine, 100 U/mL of penicillin and 100 μg/mL of streptomycin). The nucleated cells were then counted with a cytometer using 4% acetic acid. These were diluted in complete media to a concentration of 10⁵-10⁶ cells/mL, and 10 mL of the resultant solution was placed in a T75 culture flask. This was incubated for 4 days. The non-adherent cells were then aspirated in media and discarded, and the media was replaced every 3 days. At 80% confluence the culture dishes were washed with phosphate-buffered saline (PBS). The cells were treated with 3 mL of 0.5 mg/ml trypsin and 0.2 mg/ml of EDTA for 7 minutes at 37 °C. The reaction was stopped with 30 mL of complete media. The cells were split into two flasks. BM-MSC expansion was continued until the third passage. The cells were collected and centrifuged for 5 minutes at 400 x g. These cells were then resuspended and counted. The BM-MSCs (10⁶ cells/vial) were stored in complete media containing 10% DMSO and frozen by slowly decreasing the temperature to -80 °C. The cells were then stored in liquid nitrogen. Prior to application, the BM-MSCs were slightly thawed, and the frozen pellet was placed into 5 mL of complete media. This was mixed quickly until the pellets had thawed and was then plated and incubated in a T25 flask at 37 °C for 24 hours. The cells were then removed from the flask by trypsinization, centrifuged at 400 x g for 10 minutes at room temperature, and resuspended in 0.5 mL of PRP. The BM-MSCs were then used in the experiments.

**Fascial Incision and Repair**

The rats were placed under inhalational anesthesia using the EZ-AF9000 Auto Flow System anesthesia device with isoflurane (3-5%) for induction and isoflurane (1-3%) for maintenance. Time under isoflurane anesthesia ranged from 20 to 30 minutes. Respiration, tissue color, and toe pinch reflex were monitored during surgery. Rats were administered buprenorphine 0.025 mg/kg subcutaneously with the first dose prior to incision. This administration was repeated at 12 and 24 hours after surgery.
Aseptic technique and sterile surgical instruments were utilized. The midline abdominal skin was incised from xiphoid to pubis. Bilateral skin flaps were raised with sharp dissection, and a 6-cm midline full-thickness fascial incision was made. The fascia was then reaproximated with fifteen interrupted 5-0 Vicryl sutures. Group 1 received no further treatment. For the rats in group 2, each corner of a 6- x 1-cm strip of CoTa was sutured to the anterior rectus sheath using 5-0 Vicryl suture in an overlay fashion. This was followed by application of 0.5 mL of PRP to the CoTa. Group 2 received no further treatment. For group 3 rats, the same treatment was employed as for the group 2 rats except that 1 x 10^6 BM-MSCs were reconstituted in the 0.5 mL of PRP and then applied to the CoTa. The skin of each rat was then reaproximated with interrupted 5-0 Vicryl subcuticular sutures. After each procedure, the rats were placed in clean bedding and monitored during the recovery period.

**Postprocedure Monitoring**

Following surgical procedures, the rats were monitored once daily for the first week of recovery for signs of infection and autophagia. None of the study rats developed signs of clinically-significant infection or autophagia. Beyond the first recovery week, the rats were monitored two to three times weekly.

**Recovery of Fascia**

The abdominal fascia of each rat was recovered at four weeks (subgroup a) or eight weeks (subgroup b). The rats were placed under deep inhalational anesthesia as previously described. A midline sternotomy was performed. Whole blood was obtained for PRP isolation via cardiac puncture. Bilateral pneumothoraces were created. Once euthanization was complete, the cutaneous surgical scar was incised, and bilateral skin flaps were carefully raised avoiding inadvertent fascial disruption. The abdominal fascia was then incised along the subcostal and pelvic margins. The fascia was then cut using a customized cutting die fitted to a manual press. Two I-shaped segments spanning the healed fascial defect were recovered and immediately underwent stress and strength analysis. The remaining tissue was properly stored for histologic analysis.

**Specimen Analysis**

**Biomechanical Testing**

The biomechanical properties of the fascial defects for each study group were determined by standard tensiometric analysis. The tissue from each animal was cut into two dumbbell-shaped segments across the healed defect. Each group contained 7 animals with two specimens obtained per animal which yielded 14 specimens (n=14). The specimens were preserved in phosphate-buffered saline solution until the tensile strength testing. Testing was performed using an Instron Tensiometer, Model 5,500R, equipped with a 100N load cell capable of 0.25% accuracy over the entire range. Grips were machined to reduce breakage at the attachment sites. Specimens that failed at the attachment sites were not included in the final analysis.
The load cell applied a constant extension rate of 10 mm/min to each specimen until a tissue disruption occurred. Force and tissue deformation data were simultaneously recorded, and data analysis was performed with the use of the Merlin materials testing software package (Instron Corp). Data from the stretch loading was used to determine the following clinically important biomechanical properties: modulus of elasticity (stress required to strain the material 1 mm/mm in the linear region of the stress-strain curve), tensile strength (maximum stress tolerated by the tissue), and modulus of toughness (energy needed to completely rupture the tissue). Statistical analysis using ANOVA was applied to this data.

**Histologic Analysis**

The formalin-fixed specimens were embedded in paraffin. Cross-sections were taken and stained using Masson’s trichrome. Vascularity assessment was performed similar to the assessment described by Stanwix et al. Using a stereoscopic microscope at 40x magnification, two independent observers reported the total number of blood vessels at three non-overlapping fields at the incision site. Results were averaged within each group.

High-resolution photographs were taken at 40x magnification for all representative specimens (Figure 1). The images were randomized and assessed by four trained professionals for collagen organization, collagen abundance, and myocyte degeneration. These observers were blinded to individual study groups. A score of zero to three was assigned to each category using a semi-quantitative scale (Table 2).

Statistical analysis was then completed. Kruskal-Wallis tests were performed for each subgroup measuring collagen organization, collagen amount, myocyte degeneration and neovascularization. Mann-Whitney testing provided pairwise analysis between individual subgroups. Significant values (p<0.05) were tested for reliability by repeat Kruskal-Wallis analyses. Calculations were performed using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington) and the statistics add-in program, Analyze-it Standard (Analyze-it Software, Ltd., Leeds, United Kingdom).

**RESULTS**

**Biomechanical Testing**

Data from biomechanical testing was analyzed using ANOVA. Subgroup analysis was performed for each biomechanical parameter.

**Tensile strength (maximum stress)**

The fascia of the group 1 control rats had an average tensile strength of 204 kPa (range 162-246, SD 29.61) at four weeks and 421.83 (kPa 295-570, 91.5) at eight weeks (Table 3, Figures 2 and 3). As compared to group 1 rats at both time points, the group 2 rats (PRP and CoTa) had a 101% increase in average tensile strength at four weeks at 409.58 kPa (315-528.5, 67.84) and a 38% increase at eight weeks at 582.18 kPa (437-751, 94.34). Comparing group 1 to group 3 (MSCs) at both time points, the fascia of group 3 rats exhibited a 301% increase in average
tensile strength at four weeks at 817.15 kPa (654-1095, 177.74) and a 117% increase at eight weeks at 917.17 kPa (604-1184, 131.47). Group 3 had a 100% improvement compared to group 2 at four weeks and a 58% improvement at eight weeks. ANOVA analysis was performed between subgroups 1a, 2a and 3a with p<.001 between all subgroups regarding tensile strength. This was repeated for subgroups 1b, 2b and 3b with p<.001 for comparisons of all three subgroups.

**Modulus of elasticity**

At four weeks after midline fascial incision and repair, group 1 rats had an average modulus of elasticity of 1333.75 kPa (range 836.28-1653.1, SD 280.54). The average modulus of elasticity was 2178.95 kPa (1410.2-3088.2, 427.13) for group 2 rats and 5085.59 kPa (2750.4-7469.2, 1727.71) for group 3 rats. At eight weeks the average modulus of elasticity was 2865.25 kPa (1537.8-4288.6, 942.53) for group 1, 3760 kPa (2809.1-5156.5, 838.26) for group 2, and 5076.24 kPa (3256.2-6560.1, 1128.97) for group 3. ANOVA analysis was performed between subgroups 1a, 2a and 3a with p<.001 between all subgroups regarding Modulus of Elasticity. This was repeated for subgroups 1b, 2b and 3b with p<.001 for comparisons of all three subgroups.

**Modulus of toughness**

The average modulus of toughness increased between group 1 and group 2 at both four weeks (28.28 kJ/m$^3$ vs 62.65 kJ/m$^3$) and eight weeks (60.16 kJ/m$^3$ vs 111.05 kJ/m$^3$), representing an increase in maximum energy absorption of 122% at four weeks and 85% at eight weeks with the addition of CoTa and PRP alone (Table 4, Figure 4). At four weeks, the addition of MSCs (group 3) improved the total energy absorption by 320% (118.85 kJ/m$^3$, SD 37.49) compared to group 1 and by 90% compared to group 2. At eight weeks, group 3 had a 142% increase in average modulus of toughness (145.44 kJ/m$^3$, SD 28.51) compared to group 1 and a 31% increase compared to group 2. ANOVA analysis was performed between subgroups 1a, 2a and 3a with p<.001 between all subgroups regarding Modulus of Toughness. This was repeated for subgroups 1b, 2b and 3b with p<.001 for comparisons of all three subgroups.

**Histologic Analysis**

Data from the histologic analysis is summarized in Table 5. Means and standard deviations are reported for each subgroup.

**Neovascularization**

The degree of neovascularization was consistent between groups 1 and 2 at both four and eight weeks. The addition of CoTa and PRP (group 2) did not increase neovascularization compared to group 1 at either four weeks (p<0.135) or eight weeks (p<1). The addition of BM-MSCs (group 3) significantly increased neovascularization at both four weeks (p<0.001) and eight weeks (p<0.001) compared to controls (group 1). Additionally, only group 3 produced increased vascularization from four weeks to eight weeks.

**Collagen organization and amount**

Collagen organization was evaluated. Tight, parallel and less cellular collagen fibrils were deemed more organized. A cross-section of native abdominal wall fascia was used as an index. Collagen organization was not significantly different between any groups. Differences between
groups became less perceptible from four weeks (p<0.08) to eight weeks (p<0.24). Greater collagen abundance was found at four weeks (p<0.001) and eight weeks (p<0.031) in the BM-MSC group (group 3) compared to groups 1 and 2. Mean collagen abundance scores for group 3 at eight weeks (1.83) were lower than at four weeks (2.36), but these scores were higher than group 1 or 2 at either time point. Interestingly, group 3 collagen demonstrated a characteristic muscle encapsulation at the incision site in all but one rat which was not apparent in the other groups (Figure 1D).

**Myocyte degeneration**

Myocyte degeneration was less pronounced at eight weeks as compared to four weeks. The four-week group of CoTa and PRP alone (group 2a) had significantly more muscle degeneration as compared to group 1a (p<0.04) and group 3a (p<0.02).

**DISCUSSION**

The purpose of this study was to determine if the combination of all three additives, PRP, collagen, and BM-MSCs, led to an increase in tensile strength and energy absorption at four and eight weeks after implantation. There was a marked improvement when the BM-MSCs were added (group 3) when compared to both the native controls (group 1) as well as those with the vehicle alone (group 2).

Activated PRP is a known source of the required growth factors for wound healing. Platelet alpha granules release PDGF, VEGF, TGF-beta, FGF and EGF at physiologic ratios providing a ripe environment for cellular repair and proliferation. For these reasons, it seemed essential to include PRP as part of a vehicle for BM-MSC introduction into the fascial repair. However, it was also considered that due to the potential benefits of PRP, an independent effect might exist that could confound the BM-MSC results. The effect of PRP on healing wounds has shown mixed results in the current literature, but PRP added to a biologic scaffold has shown possible benefit, especially in cutaneous wound healing. Previous studies have shown that PRP placed in a wound with a dermal matrix stimulates tissue formation with increased revascularization and proliferation. Other studies have shown increased leukocyte margination and angiogenesis in wounds implanted with PRP-soaked sponges. A collagen scaffold was provided for the PRP in the fascial wound, and this combination (group 2) became the vehicle which was examined independently. In our study, group 2 had a significant increase in tensile strength and energy absorption at both four and eight weeks after fascial repair. It was expected that this would correlate with increased collagen deposition, neovascularization, and cell proliferation and migration, as has been observed in previous studies. This was not fully realized in the histologic analysis of our specimens. Histologically, although there may have been a slight increase in collagen abundance related to fibroblast proliferation in group 2 compared to group 1, it was not noted to be significantly improved. There was an increase in neovascularization from group 1 to group 2. The cellular factors contributing to increased tensile strength and energy absorption are not clear from this data. Collagen has been shown to provide a scaffold for fibroblast migration and collagen deposition which could lead to increased tensile strength and energy absorption. PRP is also known to have a proliferative effect on native fibroblasts. Further analysis of fibroblasts in the wound bed and collagen subtype and crosslinking may better explain these biomechanical improvements seen in group 2.
The addition of CoTa, type I bovine collagen, to the wound bed was based on several known characteristics which would likely positively effect wound healing. Our previous work with rabbits revealed that there was a significant absorptive capacity which would keep PRP and BM-MSCs within the wound bed. Additionally, the known macroporous nature of CoTa allows for BM-MSC adhesion and migration. Although BM-MSCs have not been directly studied in relation to CoTa, studies have demonstrated MSC, fibroblast and epithelial cell adherence and proliferation on this collagen matrix. Studies by Ui-Won Jung, et al. have shown that CoTa is mostly resorbed by eight weeks post-implantation. In our study, there was a marked increase in collagen within the scar tissue at both four and eight weeks when compared to controls. It is uncertain whether the CoTa is incorporated into this newly-formed scar tissue. The absorptive and macroporous nature of CoTa which allows for cell adherence and proliferation as well as its rapid absorption and possible incorporation into the wound bed made CoTa an ideal vehicle for BM-MSC and PRP introduction at the wound site. Group 3 rats had superior fascial healing which might be partially attributed to these unique characteristics of CoTa.

There is a strong foundation for the results of our study within the literature. BM-MSCs possess many characteristics that would theoretically aid in fascial healing. First, BM-MSCs naturally migrate to sites of wounding and produce collagen at rates superior to those of native fibroblasts. It is also known that BM-MSCs have an anti-inflammatory effect that may aid in decreasing the inflammatory phase while helping to accelerate the transition into the proliferative phase. BM-MSCs also produce a microenvironment which decreases the levels of matrix metalloproteases (MMPs) within the local milieu limiting local tissue destruction. This might reduce the need for re-creation of the local architecture for new tissue growth. This may have led to the preservation of surrounding muscle observed on histologic examination of the group 3 specimens. As opposed to preservation, at least some of this observed increase in muscle bulk may have resulted from regeneration from BM-MSCs. In either case, the improved muscle bulk in group 3 rats likely provided stability to the scar edges imparting additional strength to the abdominal wall. There is also evidence that chemotactic BM-MSCs lead to increased fibrin contraction and local strength. All of these factors may have contributed to the improved fascial healing in our study.

Although there was a marked improvement in fascial healing in group 2 without the addition of BM-MSCs, there appears to be a synergistic effect when BM-MSCs are included. There are many studies expressing an increase in proliferation of BM-MSCs with the addition of PRP. Moreover, the increased chemotactic effect of PRP on BM-MSCs is well documented. Therefore, PRP, with many important growth factors, such as PDGF and TGF-beta, likely supports the microenvironment of the healing wound allowing for increased BM-MSC proliferation within the wound and adherence to the wound. Studies also show that PRP and BM-MSCs independently lead to increased neovascularization in wounded tissues. Histological evaluation of our specimens revealed this expected increase in neovascularization in group 3, which can be attributed to one or both of these additives. Improved local neovascularization possibly accelerated improvements in local wound healing secondary to a faster recovery of local tissue hypoxia further decreasing hypoxia-induced tissue destruction.
In addition to neovascularization, there was a significant increase in collagen abundance with the addition of BM-MSCs to the fascial repair (group 3). The increase in collagen abundance compared to group 2 supports new collagen formation rather than retention of the CoTa at the repair site as the explanation for these findings. CoTa imparts no tensile strength, so the addition of CoTa could not provide the biomechanical improvements. However, collagen reorganization by the BM-MSCs could account for the increased tensile strength and energy absorption. The rapid increase in collagen found in group 3 as compared to group 1 at four weeks might be better explained by the reorganization of collagen which is already present in the form of CoTa. This is further supported by the much slower increase in tensile strength and energy absorption seen between four and eight weeks in the group 3 rats. Comparatively, the rate of increase in tensile strength and energy absorption remains fairly constant between four and eight weeks in group 1 suggesting continued new collagen deposition. Furthermore, although not statistically significant, there is a trend towards increased collagen organization in both group 3 subgroups. Multiple mechanisms, including reorganization, cross-linking of CoTa, and new collagen deposition, may explain the differences between group 1 and group 3. Further studies will be required to delineate these contributions.

Group 3 had a noticeable increase in local muscle around the scar edge. PRP alone has been shown to aid in muscle proliferation and healing related to insulin-like growth factor (IGF) and the FGF found within the PRP. While our histologic evaluation did not demonstrate this improvement in muscle survival or proliferation in group 2 (vehicle only), this was seen in group 3. It is possible that the BM-MSCs combined with the PRP synergistically improved muscle survival and proliferation. While the trophic effects of PRP in the local environment, especially in relationship to BM-MSCs, seem to lead to improved fascial healing, the supportive effect of PRP on BM-MSCs is not clear in this study. It is likely that the combination of BM-MSCs, PRP and CoTa further optimizes fascial repair throughout the entire inflammatory and proliferative phase of wound healing.

The effects of BM-MSCs on fascial healing have been studied by McFarlin, et al. previously. In this study BM-MSCs were injected into the tail veins of rats which had a repaired fascial defect, and the wound repairs were tested at seven and fourteen days. In another study group BM-MSCs were directly injected over the repaired fascial defect, and the fascia was analyzed at only seven days. This study showed a two-to-three-fold increase in wound breaking strength at seven days with these improvements already diminishing at fourteen days as compared to controls. Our study resulted in a four-fold increase in tensile strength and energy absorption (modulus of toughness) at four weeks and a more than two-fold increase at eight weeks. BM-MSCs were only directly applied to the fascia in our study as systemically-administered BM-MSCs are preferentially sequestered by the lungs. Also, our study utilized only 1x10^6 BM-MSCs while 6x10^6 MSCs were applied to the wound bed and 8x10^6 BM-MSCs were injected systemically in the study by McFarlin et al. The improved results in our study, despite a six- to eight-fold decrease in the number of BM-MSCs, are probably realized due to the combination of biologic and cellular additives that was used. Further testing at earlier and later time points is required.

Conclusion
This study shows that the addition of these cells to platelet-rich plasma and CollaTape™ leads to a marked improvement in abdominal wall strength and energy absorption. From a histologic perspective, there is also an improvement in muscle survival along the scar edges with improved collagen abundance and neovascularization. The combination of these findings directly relates to the increase in abdominal wall strength. Future work applying this combination therapy will provide insight into its utility in various disease models. Studies involving diabetes, obesity, and immunocompromise will further evaluate if BM-MSCs combined with PRP and CollaTape™ will truly improve fascial repair in high-risk populations. Continued research in this field will hopefully lead to a cell-based therapy to minimize postoperative hernia formation and its resulting morbidity.

REFERENCES


### Table 1. Study Design

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Technique of Closure</th>
<th>Time to Sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Midline abdominal incision with primary suture repair</td>
<td>4 Weeks</td>
</tr>
<tr>
<td>1b</td>
<td>Midline abdominal incision with primary suture repair</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>2a</td>
<td>Same as Group 1 + PRP + CoTa</td>
<td>4 Weeks</td>
</tr>
<tr>
<td>2b</td>
<td>Same as Group 1 + PRP + CoTa</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>3a</td>
<td>Same as Group 2 + MSCs</td>
<td>4 Weeks</td>
</tr>
<tr>
<td>3b+</td>
<td>Same as Group 2 + MSCs</td>
<td>8 Weeks</td>
</tr>
</tbody>
</table>

*One animal died during initial surgery from anesthesia (n=6), all other subgroups (n=7). PRP=platelet-rich plasma, CoTa=CollaTape™, MSCs=mesenchymal stromal cells.

### Table 2. Histological Scoring Criteria

<table>
<thead>
<tr>
<th>Category</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen Organization</td>
<td>Disorganized</td>
<td>Mildly Organized</td>
<td>Moderately Organized</td>
<td>Well Organized</td>
</tr>
<tr>
<td>Collagen Amount</td>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>Abundant</td>
</tr>
<tr>
<td>Myocyte Degeneration</td>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>Abundant</td>
</tr>
</tbody>
</table>
**Table 3.** Maximum Stress (kPa)

<table>
<thead>
<tr>
<th>Group</th>
<th>N*</th>
<th>Mean**</th>
<th>Standard Deviation</th>
<th>Minimum Value</th>
<th>Maximum Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>13</td>
<td>204.00</td>
<td>29.61</td>
<td>162</td>
<td>246</td>
</tr>
<tr>
<td>1b</td>
<td>12</td>
<td>421.83</td>
<td>91.55</td>
<td>295</td>
<td>570</td>
</tr>
<tr>
<td>2a</td>
<td>13</td>
<td>409.58</td>
<td>67.84</td>
<td>315</td>
<td>529</td>
</tr>
<tr>
<td>2b</td>
<td>11</td>
<td>582.15</td>
<td>94.34</td>
<td>437</td>
<td>751</td>
</tr>
<tr>
<td>3a</td>
<td>13</td>
<td>817.15</td>
<td>177.74</td>
<td>604</td>
<td>1184</td>
</tr>
<tr>
<td>3b+</td>
<td>12</td>
<td>917.17</td>
<td>131.47</td>
<td>654</td>
<td>1095</td>
</tr>
</tbody>
</table>

Two specimens for biomechanical testing were taken from each animal.

*One animal died during initial surgery from anesthesia.

*Specimens that failed at the grips were not included.

**Data analyzed using ANOVA within subgroup a and with subgroup b (p<0.001).

**Table 4.** Modulus of Toughness (kJ/m$^3$)

<table>
<thead>
<tr>
<th>Group</th>
<th>N*</th>
<th>Mean**</th>
<th>Standard Deviation</th>
<th>Minimum Value</th>
<th>Maximum Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>13</td>
<td>28.28</td>
<td>8.12</td>
<td>15.76</td>
<td>42.52</td>
</tr>
<tr>
<td>1b</td>
<td>12</td>
<td>60.16</td>
<td>25.39</td>
<td>23.82</td>
<td>111.29</td>
</tr>
<tr>
<td>2a</td>
<td>13</td>
<td>62.65</td>
<td>19.55</td>
<td>33.44</td>
<td>104.08</td>
</tr>
<tr>
<td>2b</td>
<td>11</td>
<td>111.05</td>
<td>51.57</td>
<td>42.01</td>
<td>216.56</td>
</tr>
<tr>
<td>3a</td>
<td>13</td>
<td>118.85</td>
<td>37.49</td>
<td>63.20</td>
<td>182.22</td>
</tr>
<tr>
<td>3b+</td>
<td>12</td>
<td>145.44</td>
<td>28.51</td>
<td>113.05</td>
<td>212.51</td>
</tr>
</tbody>
</table>

Two specimens for biomechanical testing were taken from each animal.

*One animal died during initial surgery from anesthesia.

*Specimens that failed at the grips were not included.

**Data analyzed using ANOVA within subgroup a and with subgroup b (p<0.001).
Table 5. Data Summary

<table>
<thead>
<tr>
<th>Groups</th>
<th>Neovascularization</th>
<th>Organization</th>
<th>Amount</th>
<th>Degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>2.0 (1.3)</td>
<td>1.21 (0.96)</td>
<td>1.11 (0.69)</td>
<td>1.32 (1.06)</td>
</tr>
<tr>
<td>1b</td>
<td>1.5 (0.8)</td>
<td>1.46 (1.04)</td>
<td>1.32 (0.86)</td>
<td>0.93 (0.66)</td>
</tr>
<tr>
<td>2a</td>
<td>1.4 (1.1)</td>
<td>1.11 (0.69)</td>
<td>1.57 (0.74)</td>
<td>1.93 (0.94)*</td>
</tr>
<tr>
<td>2b</td>
<td>1.5 (0.7)</td>
<td>1.07 (0.86)</td>
<td>1.25 (0.80)</td>
<td>1.18 (0.94)</td>
</tr>
<tr>
<td>3a</td>
<td>4.2 (2.3)*</td>
<td>1.57 (0.63)</td>
<td>2.36 (0.73)*</td>
<td>1.32 (0.77)</td>
</tr>
<tr>
<td>3b*</td>
<td>5.9 (2.8)*</td>
<td>1.42 (0.88)</td>
<td>1.83 (0.82)*</td>
<td>0.96 (0.75)</td>
</tr>
</tbody>
</table>

*P <0.05 on the basis of Mann-Whitney paired analysis and Kruskal-Wallis group analysis.

*One animal died during initial surgery from anesthesia (n=6), all other groups (n=7).

Data is given as means and standard deviations for all groups.
Figure 1. Trichrome stain at 40x magnification. (A) Native abdominal wall centered at the linea alba. Representative histology for group 1 (B), group 2 (C) and group 3 (D) at 4 weeks centered at the incision site. Group 3 demonstrates greater collagen abundance and intramuscular collagen deposition.
Figure 2. Box and whisker plot of tensile strength (maximum stress). There is no overlap in data points between any of the four-week subgroups. There is also no overlap in data points between the control group and the MSC (mesenchymal stromal cell) group at eight weeks.
Figure 3. Average stress-strain curves. The data points were averaged to create a curve of best fit for each subgroup. The maximum height of each curve is the maximum stress (tensile strength). The area under the curve up to the point of failure is the modulus of toughness, or energy absorbed by the tissue.
Figure 4. Bar graph of modulus of toughness. The modulus of toughness approximately doubles between each group at four weeks. ANOVA was performed for means of subgroups 1A, 2A and 3A with p<.001 this was repeated for subgroups 1B, 2B and 3B with p<.001.