Healing the body using the patient’s own cells.

Large OCD in knee

One year later
Regenerative Science Inc. (RSI)
History and Research and Development timeline

The timeline above described the steps leading to the Regenexx™ Procedure.

In more details, the pre-clinical data with several independent papers published in the literature by independent world-known groups report the use of MSCs to repair full-thickness, joint cartilage defects, tendon repair, bone defect of critical size in large animal models (Reviewed by Caplan et al., 2005 and Krampera et al., 2006)

RSI initiates a safety study with autologous adult bone marrow-derived cells for the treatment of orthopedic injuries, under an IRB from the Spinal Injury Foundation (IRB00002637) with 45 patients. Based on the very favorable safety profile of the procedure (Centeno et al., 2010), RSI starts treating patients in 2007 with the newly developed Regenexx™ Procedure.

Other international groups begin to publish case reports of bone marrow derived cells used to treat orthopedic condition (Wakitani et al., 2002, Wakitani et al., 2004, Matsumoto et al, 2010)

Caplan A. I., 2005. Tissue Eng 11, 1198
Krampera M. et al., 2006. Bone 39, 678
Centeno C. J. et al., 2010. Curr Stem Cell Res Ther 5, 81
Wakitani S., K. et al., 2002. Osteoarthritis Cartilage 10, 402
Wakitani S. et al., 2004. Cell Transplant 13, 595
Pre-clinical/Animal studies

Rat model of a femoral segmental defect treated with bone marrow derived mononuclear cells

**Methods:** adult male Lewis syngeneic rats underwent a unilateral operation to create a 5 mm defect in the mid-femoral diaphysis, equal to 2.5 times the cross sectional diameter (2.0 mm; volume, 0.037 cc).

**Results:** Radiograph at 12 weeks demonstrating) a nonunion in the control group (control) and a united defect with remodeled cortex in the group implanted with 0.075cc bone marrow (volume equal to twice the volume of the defect). (Werntz JR et al. 2006 Journal of Orthopaedic Research)

Rabbit cartilage defect treated with bone marrow mononuclear cells

**Methods:** 7 days after large full-thickness cartilage defects were created, fibrin gel with or without autologous uncultured bone marrow-derived mononuclear cells were transplanted in the articular cavity

**Results:** (1) neo-regeneration of cartilage; (2) large production of Type II collagen, and (3) histological findings and morphometrical measurements showed that the BM-MNC group had superior cartilage repair compared with the control group. (Chang F et al. 2007 Journal of Orthopaedic research)

Goat knee OA treated with bone marrow-derived expanded MSCs

**Methods:** OA induced unilaterally in the knee joint by complete excision of the medial meniscus and resection of the anterior cruciate ligament. After 6 weeks, 10 million autologous cells was delivered by direct intraarticular injection.

**Results:** The cell treatment resulted in the formation of tissue in the posterior compartment at 12 weeks after complete medial meniscectomy. The excised tissues (B and C) inserted between the distal head of the femur and proximal tibial plateau (A) are shown. Regenerated hyaline-like meniscal tissue is also evident at 20 weeks after MSC injection (D and E). The regenerated meniscal horn protected the posterior tibial plateau (D) and had reformed a functional enthesis with the tibial bone via the meniscal insertion ligament (arrow in E). (Murphy JM et al. 2003 Arthritis and Rheumatism)

Dog non-union bone defect treated with bone marrow derived expanded MSCs

**Methods:** Dogs had a unilateral resection of a 21 mm long osteoperiosteal segment of the femoral diaphysis. The defect was either not filled or filled with a ceramic cylinder (scaffold) with or without autologous bone marrow derived expanded mesenchymal stem cells.

**Results:** radiographs of a segmental defect that had been left untreated (no ceramic cylinder) and little of no new bone formed and non union occurred. While treated with a ceramic cylinder (scaffold), union occurred at the host bone-implant interfaces, but bone formation did not occur throughout the entire implant. When treated with a scaffold loaded with expanded autologous mesenchymal stem cells (MSCs), union at the host bone-implant interfaces is evident, and a substantial callus formed with a thickness reaching maximum at 12 weeks for that dog. (Bruder SP et al. 1998 The Journal of Bone and Joint Surgery)
The Regenexx™ Procedure is a breakthrough, minimally invasive alternative to standard orthopedic surgical solutions to treat bone and joint injuries and diseases. Prospective patients seek alternatives to surgery which include decreased risk of related adverse events, are minimally invasive, and cost sensitive in the long term.

A key to the success of the Regenexx™ Procedure is the holistic evaluation of the patient which incorporates general health, current medications, activity level in addition to diagnosis.

For a patient that elects to undergo the Regenexx™ Procedure, 60cc of bone marrow is collected using C-arm fluoroscopy under local anesthesia. The bone marrow aspirate provides the patient’s own stem cells that are then formulated and/or expanded using growth factors present in the patient’s own blood (the process is completely autologous and done following strict sterility guidelines).

The cells are re-injected using C-arm fluoroscopy to place the cells at the damaged area using topical anesthesia.
Safety Profile of the Regenexx™ Procedure exceeds arthroplasty

A total of 227 patients followed up from 3 months to 3 years, several of whom received multiple re-injections. 29 patients (12.7%) reported adverse events; only 13 (5.7%) of these were possibly related to the Regenexx™ procedure, mostly mild to moderate pain treated with standard of care (Centeno et al., 2010).

In comparison, a retrospective study of more than 17,000 total knee arthroplasties (TKA) demonstrated that the frequency of serious surgical complications was 7.7% with a frequency of re-admission for any reason within 90 days of 8.3% (Khatod, 2008).

Centeno C. J. et al., 2010. Curr Stem Cell Res Ther 5, 81
There are currently two cell processing alternatives for the Regenexx™ Procedure:

1. The same day, or SD, uses the buffy coat obtained after centrifugation of the bone marrow plus other blood-derived factors. That buffy coat contains hematopoietic progenitor cells, mesenchymal progenitor cells and other mononuclear cells. This is similar to the process yielding BMAC.

2. The full expansion procedure starts after the mesenchymal stem cells (MSC) are isolated from the bone marrow. Ultimately, MSCs from a single sample are expanded 100 fold. After re-injection, greater cell numbers are thought to favor a more rapid and thorough repair of the damaged area.

MSCs are cells able to differentiate into bone, cartilage, tendon, or fat in vitro. In vivo, MSCs receive differentiation cues from their environment. For example, stretching or pulling forces can cause MSCs to become tendon cells. Compressive loading like walking can pushes MSCs towards cartilage lineage. Therefore, to maximize patients outcomes after the Regenexx™ Procedure, limited activity such as walking is encouraged.

Another clinically meaningful feature of MSCs is their ability to reduce inflammation, thus favoring repair processes that limit scar tissue formation.
Regenexx™ Procedure: Same day (SD)

SD cell processing protocol yields a product rich in both HSCs and MSCs. HSCs have been shown to promote revascularization (Rafii et al, 2002). As a result, applications that required less MSCs (such as a small joint) or neo-vascularization (such as meniscus repair or generalized osteoarthritis) would be more responsive to the Regenexx™ SD Procedure.

The safety profile of MB-MNCs is proven by 15+ years of practice in orthopedic (Hernigou, 2009 and Gangji, 2010) as well as in other applications such as myocardial infarction (reviewed by Abdel-Latif, 2009).

The best indication appears to be symptomatic hips with osteonecrosis without collapse. Some patients with limited joint or tissue damaged may also prefer a one day Regenexx™ SD procedure.

RSI has also developed a proprietary process to extract growth factors (Super Platelet Mix) from the patient’s own blood that better supports MSCs growth than platelet lysate or PRP. The bone marrow-derived cells are formulated in the proprietary Super Platelet Mix during the re-injection process.

Abdel-Latif A. et al., 2007. Arch Intern Med 167, 989
Hernigou P. et al., 2009. Indian J Orthop 43, 40
The Expanded Cells procedure requires about 7 weeks between harvest and re-injection to allow for cell culture and quality control. This process generates a wholly autologous (no animal byproduct) enriched cell population optimized for those indications that require new tissue formation.

### Cells Characteristics

- **Mesenchymal stem cells (MSCs)** present a fibroblastic morphology when adherent to plastic (see below)

- **Surface markers**
  - Our cells are characterized using standard surface markers, and have the following phenotype CD31-, CD34-, CD44+, CD73+, CD90+, and CD105+.

- **Less than 21 days in culture.**
  - Human mesenchymal stem cells in culture show progressive growth arrest and entered senescence without evidence of transformation (Tarte et al., 2009). However, their doubling time reduces after the 7th passage. Consequently, our cells are expanded for <21 days (3 passages for slow growers, 4 for fast growers). In addition, the cells are tested for the presence of chromosomal abnormality by karyotype; a technique that literally counts the number of chromosomes per cells.

- **Yields $10^7$ MSCs**
  - The average amount of MSCs recovered from a bone marrow aspirate (measured by their abilities to form colonies) varies tremendously depending of the patient with an average of 500-5000 CFU per ml of bone marrow aspirate. In 21 days, the MSCs expand to $5 \times 10^6$ to $10^8$ MSCs, an average of 100 fold their original number.
Regenexx™ Procedure: Collection and Re-Injection Procedure

Fluoroscopy is used to guide the collection of the bone marrow aspirate (Picture below, on the left) and the re-injection of the cells to a specific site in the joint or disc in most need of repair. This method of re-implantation avoids invasive surgery (picture below, on the right) and has shown high potency for the medial meniscus of the knee or the right posterior-lateral portion of the L5-S1 disc, for example.

One or more injections are delivered based on the clinical indication and the patient’s response to the treatment.

The cells are formulated for injection with platelet-derived growth factors to enhance engraftment, as all cells demonstrated significant proliferation in autologous platelet derived growth factors in test culture.

Fluoroscopy pictures from Centeno C. J. et al., 2008. Pain Physician 11, 343
Summary of indications developed by Q3-2010

1- Peripheral joints
   • Knee patients
     • Survey of 155 knee patients
     • 5 examples of Case Study
   • Hip patients
     • Survey of 70 hip patients
     • 1 example of Case Study
   • Ankle patients
     • 2 examples of Case Study

2- Lumbar discs
   • L5-S1 patients
     • 3 examples of Case Study

3- Fracture Non-union
   • 1 example of Case study

4- Tendons ligaments
   • Disrupted talo-fibular ligament patient
     • 1 example of Case Study

Other indications in development...
Peripheral joints

• Knee patients
  • Treatment characteristics
  • Survey of 155 knee patients
    • % change in pain
    • FRI and VAS test
  • 5 examples of Case Study
Can treat: Osteochondral defects causing pain, or generalized knee OA.

Our own outcome research shows patients with severe OA (bone spurring, multiple compartment involvement) have less robust outcomes than patients with uni-compartmental OA with mild bone spurring or isolated OCD’s.

**Best indications:**
- Chronic OCD
- Meniscus tearing
- Uni-compartmental osteoarthritis

**Best patient demographics:**
- Men/women from 18 to 81 year old
- Healthy (limited amount of medication)
- Active (better outcomes)

70% men - 30% women
Age 18-81
BMI 18-37
Survey of 155 knee patients treated with the Regenexx™ Procedure (Expanded Cells)

The 155 patients were followed from 3 months to 2 years with an average 37% lost to follow up; several of the Regenexx™ patients live outside of the US.

• Of the patients that responded to the follow up questionnaire, 40/61 patients reported an reduction in the amount of pain (% of change) of 50% or greater at 12 months follow up.

• Although the majority of these patients were candidates for knee replacement, only 6 patients underwent surgery (replacement or other).

Compare to standard of care (untreated group), the reduction in pain experienced by the patients receiving the Regenexx™ Procedure, is striking.

Overtime, the knee patient population is reporting reduction in pain (higher % change)

* Number of patients who responded at each time point (n for treated, C for untreated)

**Include knee replacement or knee resurfacing
Survey knee patients: FRI (function) and VAS (pain) test

For this FRI test, before (B) and at least 6 months after (A) the Regenexx™ Procedure, the patients answered a questionnaire with a series of questions corresponding to each category (pain intensity, sleep, etc). The answers are then combined into a score (0, 1, 2, 3, and 4); 0 being a positive outcome while 4 is a negative.

As expected the scores between before and after for activities that are directly linked to the knee were highly statically significant: pain intensity ($P=0.003$) or walking ($P=0.008$), while other measures such as lifting, which does not rely on knee function, was not significantly changed.

Similar results were obtained with the VAS test. The table shows the $p$ value for the difference between before and at least 6 months after the Regenexx™ Procedure.

<table>
<thead>
<tr>
<th>VAS test results:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Least Pain</td>
<td>0.3933</td>
</tr>
<tr>
<td>Worst pain</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Average Pain</td>
<td>0.0011</td>
</tr>
<tr>
<td>How Often (frequency)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>How Long (duration)</td>
<td>0.0094</td>
</tr>
</tbody>
</table>

Conclusion: The Regenexx™ Procedure reduces significantly the pain in treated knee patients
Case studies

Patient K1: 46 year old Caucasian woman

Diagnosis/previous treatments: Large OCD who failed arthroscopic debridement

Results: The white bracket outlines the cartilage layer in this Sagittal MRI. The red arrows point to the OCD (hole in the cartilage-dark area among the lighter cartilage). The yellow arrows point to the defect one year after the Regenexx™ knee Procedure. Note that the dark area is much less in the after image and for the most part have been “filled in”.

At the two year follow-up, the patient continued to report marked subjective improvement.

Imaging: Pi Gr-Top images are pre-op in Jan 07-3D FSPGR Sag -ET=1, TR=9.4, TE=4.4. Bottom images are Jan 08-3D FSPGR Sag -ET=1, TR=9.4, TE=4.4.
Patient K2: 34 years old Caucasian man

**Diagnosis/previous treatments:** history of complex medial meniscus tearing times several years

**Results:** The meniscus is the triangular structure in the dashed circles. This view of the meniscus shows the bright lines in the dark meniscus are tears in that structure. Note the decrease in bright lines in the after films indicates consolidation of the tearing. The patient reported excellent relief of chronic medial knee pain with physical activity.

**Imaging:** Proton density fast spin images with a 3.0 T MRI
Patient K3: 51 years old Caucasian woman

Diagnosis/previous treatments: Chronic pain and failed arthroscopic debridement with pain on walking long distances and hiking.

Results: These images scan across the area of the knee with obvious defects in the grey cartilage on the darker bone. The red arrows on the “Before” images show breaks in the grey cartilage (whiter areas in the grey). The bottom images are 16 months after the Regenexx™ knee Procedure. The yellow arrows point to the same areas which have now “filled in” with grey cartilage (no more white areas).

The patient regained the ability to pursue long hikes.

**Patient K4:** 37 years old Caucasian man

**Diagnosis/previous treatments:** chronic knee pain and partial thickness cartilage loss

**Results:** “Before” image is 5 months prior first MSC transplant. “After” is 3 months post 2\textsuperscript{nd} MSC transplant. Thicker chondral cartilage in bottom after images, generally 1 mm thicker (25-33\% thicker) than before.

Patient K5: 54 years old Caucasian woman

**Diagnosis/previous treatments:** Small lateral compartment OCD

**Results:** Survey of coronal slices across the lateral compartment. The cartilage layer is the light grey within the dashed circles. The bright white areas in the red dashed before images show “holes” in the cartilage. The yellow dashed lines encircle the same area that now has more normal grey cartilage appearance on this MRI image. Comparison of the MRI indicates 70% improvement approximately 1 year after procedure.

The patient returned to competitive horse jumping.

**Imaging:** Matching 3.0T COR PDFS images, before is May 08 and after is Jun 09. Pre is ET:7, TR:3466.7, TE: 47.5. Post is ET:7, TR: 3466.7, TE: 47.6.
Peripheral joints

• Knee patients
  • Survey of 155 knee patients
  • 5 examples of Case Study

• Hip patients
  • Treatment characteristics
  • Survey of 70 hip patients
  • 1 example of Case Study
Survey of the hip patients treated with the Regenexx™ Procedure

Best indications:
- Mild to moderate OA
- No evidence of hip effusion
- Preserved ROM of the hip

Patient demographics:
- 70% men - 30% women
- Age 21-88
- BMI 18-39

From a prospective survey of patients treated for hip injuries, 16/28 patients reported a decrease in the amount of pain (% of change) of 50% or greater at the 6 month follow up with a median of 50% improvement among the group. At one year follow up, 50% of the patients report at least 75% reduction in the amount of pain, while ~90% of the patients report at least 50% pain reduction.

Compare to standard of care (untreated group), the change in amount of pain in the patients receiving the Regenexx™ Procedure is remarkable.

**Survey of the hip patients treated with the Regenexx™ Procedure**

![Graph showing % change for hip patients](image)

**Demographics of the Regenexx Hip Patients**

![Demographics graph](image)

- **25 or 75% percentile**
- **Median**
- **Patient reported % change**
- **Number of patients who responded at each time point (n for treated, C for untreated)**
- **Include hip replacement or other type of surgeries**
Case study

**Patient H1:** 64 year old Caucasian man

**Diagnosis/previous treatments:** 20 year history of unilateral hip pain that had become debilitating over the last several years. MRI of the affected hip showed severe degeneration with spurring, decrease in joint space, and several large subchondral cysts.

**Results:** The pre-Regenexx™ Procedure image shows a discontinuous joint surface with no clearly identifiable joint space at the superior lateral weight bearing surface. The 4 week post-procedure image below demonstrates a clearly identifiable joint space in this same region with the area above the dominant subchondral cyst now demonstrating apparent neocortex.

Centeno C. J. et al., 2006. Pain Physician 9, 253
Peripheral joints

• Knee patients
  • Survey of 155 knee patients
  • 5 examples of Case Study

• Hip patients
  • Survey of 70 hip patients
  • 1 example of Case Study

• Ankle patients
  • 2 examples of Case Study
Case studies

Patient A1: 46 year old Caucasian woman

Diagnosis/previous treatments: Failed talar dome debridement for chronic OCD.

Results: The pre-Regenexx™ Procedure image shows a yellow dashed line with a “white area” that represents a “hole” in the normal grey cartilage in this coronal image of the ankle. The lighter color in the white dashed circle is swelling in the bone due to the hole in the cartilage allowing forces to be transmitted to bone. The yellow dashed circle becomes filled with normal grey cartilage after the stem cell injection and the bone color in the dashed white circle becomes uniformly dark, indicating that the abnormal bone swelling has remitted.

Patient A2: 30 years old Caucasian man

Diagnosis/previous treatments: Congenital absence of the fibula and a dysplastic foot with chronic pain in the talar dome region

Results: The lighter area at the end of the yellow arrow in the “before” image indicates a hole in the dark grey cartilage. The fact that the light area has for the most part resolved by the “after” image (end of yellow arrow) indicates improvement in the cartilage after the Regenexx™ Procedure.
1- Peripheral joints
   • Knee patients
   • Hip patients
   • Ankle patients

2- Lumbar discs
   • L5-S1 patients
     • Best indications and Demographics
     • 3 examples of Case Study
Best indications:

• Contained disc bulge pressing on a spinal nerve
• Good preservation of at least 75% of disc height

Best patient demographics:

• Patient under 50 years of age
Patient D1: 32 year old Caucasian man

Diagnosis/previous treatments: many years of history of chronic lumbar radiculopathy due to central L5-S1 disc bulge causing compression of bilateral S1 nerve roots.

Results: 3 month follow up MRI on right shows significant reduction in the size of disc bulge. The patient reported elimination of leg pain at 3 months after the Regenexx™ Procedure.
Patient D2: 39 year old Caucasian male

Diagnosis/previous treatments: 16 year history of low back and leg pain from a lifting injury

Protocol: Regenexx™ Expended Cells Procedure re-injected into the L5-S1 disc

Results: 7 months after the Regenexx™ Procedure, the MRI shows significant reduction in size of disc bulge

Imaging: Pre-Regenexx™ Procedure: 3.0 T GE MRI ET=16, TR=5350.0, TE=99.6, Post-procedure: ET=16, TR=5483.3, TE=103.0

Imaging: Pre-Regenexx™ Procedure: 3.0 T GE MRI ET=16, TR=2966.7, TE=108.4, Post-procedure: ET=16, TR=3016.7, TE=106.8
**Patient D3:** 39 year old Caucasian man with Significant improvement in back and leg pain 1 month post procedure.

**Diagnosis/previous treatments:** 16 year history of progressive low back and leg pain.

**Results:** Note the white dashed line drawn along the back of the vertebral body of L5 and the sacrum. In the before image (red dashed circle), a much larger disc bulge is seen (the material to the right of the line), versus in the post-Regenexx™ Procedure image (yellow dashed circle).


**Results:** In the after image, the yellow arrow points to the area where the bulge past the disc margin (white dashed circle) is not longer present.

1- Peripheral joints
   • Knee patients
   • Hip patients
   • Ankle patients

2- Lumbar discs
   • L5-S1 patients

3- Fracture Non-union
   • Best indications
   • 1 example of Case study
Fracture Non-union

Can treat: Non-healing fractures that have failed conservative management with intact stabilization (usually ORIF).

Best Indications:

- Delayed or non-union
- Gap between fragments not exceeding 1 cm
- Stable fracture (existing ORIF or otherwise stabilized easily with immobilization)
Case study: Fracture non-union

Patient NU1: 38 year old Caucasian woman

Diagnosis/previous treatments: 9 month old non-union who had failed a trial of a bone stimulator.

Results: Regenexx™ Procedure expanded cells implanted under fluorography guidance into fracture lucency. After radiograph is 5 weeks after MSC transplant.
1- Peripheral joints
   • Knee patients
   • Hip patients
   • Ankle patients
2- Lumbar discs
   • L5-S1 patients
3- Fracture Non-union

4- Tendons ligaments
   • Best indications
   • Disrupted talo-fibular ligament patient
     • 1 example of Case Study
Tendons/Ligaments

Can treat: Partial thickness to small full thickness tears. Large tears with severe retraction can not be successfully treated at this time.

Best indications:

• Partial or full thickness tear
• No evidence on high field MRI of retraction
• Area easily accessible with imaging guidance (fluoroscopy or MSK ultrasound)
Case study: disrupted talo-fibular ligament

**Patient L1:** 32 year old Caucasian woman

**Diagnosis/previous treatments:** Several year history of significant ankle pain from a fall. The patient had failed arthroscopic debridement, steroids, prolotherapy, and physical therapy and still had chronic ankle pain.

**Results:** Note the partially disrupted talo-fibular ligament (in dotted white circle) on the left on the January, 2008 pre-op image. The same ligament in the May 2008 MRI shows that the upper portion of the ligament has repaired and that the “crimped” appearance of the sub failure stretch injury present in the left image has returned to the more normal morphology.

The patient had complete resolution of lateral ankle pain.

**Imaging:** Pre and 3 month post MSC transplant COR PDFS 3.0T MRI of the lateral ankle ligaments and talardome.
Summary

The Regenexx™ Procedure meets the criteria of the patient’s wish list:

- Safe
- Reduces pain
- Minimally invasive
- Limited downtime
- Cost effective

The Regenexx™ Procedure meets the criteria of the physician’s wish list:

- Safe
- Reduces pain/repair injuries
- No long term negative effects
- Autologous process
- Reproducible
- Efficacy proven in elderly patients
Supplemental pre-clinical data

**Selected animal related studies**

- Chang F *et al.* 2007 *Journal of Orthopaedic research*
- Arinzeh TL *et al.* 2003 *The journal of bone and joint surgery*
- Toghraie FS *et al.* 2010 EPub
- Murphy JM *et al.* 2003. *Arthritis & Rheumatism*
Animal studies: Bone marrow derived mononuclear cells to treat cartilage defects

Rabbit cartilage defect treated with bone marrow mononuclear cells

Methods: After fixing with a hinged external fixator, the entire surface of the left tibial plateau was resected and large full-thickness cartilage defects were formed in 48 rabbits. Animals were divided into four groups: autologous uncultured bone marrow-derived mononuclear cells with fibrin gel, autologous uncultured peripheral blood-derived mononuclear cells with fibrin gel, fibrin gel alone, or nothing transplanted to the articular cavity 7 days after the operation.

Results: Histological findings showed that the bone marrow cell group had superior cartilage repair compared with the other groups, and that the peripheral blood cell and control group showed better cartilage repair than did the fibrin gel alone group.

Photomicrographs showing repair of cartilage in the bone marrow cell group (a, e, l, m), the peripheral blood cell group (b, f, j, n), the control group (c, g, k, o), and the fibrin gel group (d, h, i, p), 8 (a–d) and 12 (e–p) weeks after the operation. Safranin-O staining (a–h) and immunohistochemical staining of type I (m–p), type II (l–l) collagen were carried out (original magnification 100).

Chang F et al. 2007 Journal of Orthopaedic research
Animal studies: Expanded MSCs to treat bone defect

Dog non-union bone defect treated with bone marrow derived MSCs

Methods: A critical-sized segmental bone defect, 21 mm in length, was created in the mid-portion of the femoral diaphysis of twelve adult dogs that weighed between 22 and 25 kg. Each defect was treated with bone marrow derived allogeneic mesenchymal stem cells loaded onto a hollow ceramic cylinder consisting of hydroxyapatite-tricalcium phosphate.

Results: Radiographs, made at sixteen weeks, of segmental defects that were left untreated, defects treated with cell-free hydroxyapatite-tricalcium phosphate, and defects treated with autologous mesenchymal stem cells (MSC) loaded onto hydroxyapatite-tricalcium phosphate. The empty (untreated) defect remained unhealed. Union at the host bone-implant interfaces occurred for the cell-free implants, but the bone defect appeared granular and fractured. Defects treated with autologous mesenchymal stem cells loaded on hydroxyapatite and tricalcium phosphate implants were bridged with radiodense tissue.

Photomicrographs of defects treated with allogeneic mesenchymal stem cells loaded onto hydroxyapatite-tricalcium phosphate (C) implants (middle section, hematoxylin and eosin, magnification ×100). (a) At four weeks after implantation, mostly loose connective tissue was seen in the middle section of the defect occupying the porous space of the implant. Woven bone (WB) was detected in scant amounts in the central pores of the implant. (b) At eight weeks, woven and mature lamellar bone (B) was detected in the pore space of the implant.

Arinzech TL et al. 2003 The journal of bone and joint surgery
Animal studies: Expanded MSCs to treat OA

Goat OA treated with bone marrow derived MSCs

Methods: OA was induced unilaterally in the knee joint of donor animals by complete excision of the medial meniscus and resection of the anterior cruciate ligament. After 6 weeks, a single dose of 10 million autologous bone marrow derived MSCs suspended in a dilute solution of sodium hyaluronan was delivered to the injured knee by direct intraarticular injection. Control animals received sodium hyaluronan alone.

Results: Microscopic analysis of the medial femoral condyle. Goat knee joints were subjected to total medial meniscectomy and anterior cruciate ligament resection, which was followed 6 weeks later by an intraarticular injection of hyaluronan (A–F) or mesenchymal stem cells (MSCs) resuspended in hyaluronan (G–P). Osteoarthritic changes such as proteoglycan depletion (indicated by a reduction in surface staining), severe fibrillation, and loss of cartilage, and osteophytes (Os) and bone remodeling were evident in vehicle-treated joints (A–E). Large chondrocyte clone (arrows) were evident in areas distant to the primary lesion (F). Boxed areas in A, C, and E are shown as expanded images in B, D, and F, respectively. In joints that demonstrated evidence of meniscal regeneration after MSC application (G–N), changes to the cartilage and bone were much less severe. Mild surface roughening (H) and proteoglycan depletion in the surface zone (H–N) were evident. Proliferation of cells at the cartilage surface was also seen (L and N). Boxed areas in G, I, K, and M are shown as expanded images in H, J, L, and N, respectively.

Microscopic analysis of regenerated meniscal tissue. Green fluorescent protein (GFP)–positive cells were detected primarily at the surface (B and C) and also in the center (D) of neomeniscal tissue 6 weeks after injection of GFP-transduced mesenchymal stem cells. Neomeniscal tissue not exposed to the joint environment was used as a negative control (A).

Immunohistochemical staining of the posterior meniscal-like tissue indicated a dense, cellular, type I collagen positive fibrous network (results not shown) with small areas of more rounded cells that were type II collagen positive (E and F). By 20 weeks after injection, the neomeniscus had areas of Safranin O–positive proteoglycan and type II collagen (G and I, respectively) in a type I collagen background (H). Further analysis of the type II collagen staining (boxed area of I) showed the typical appearance of fibrocartilage (J). (Original magnification 200 in A–D; 20 in G–I; 100 in E, F, and J.)

Methods: OA was induced by unilaterally anterior cruciate ligament transection of knee joints. Twelve weeks after operation, a single dose of 1 million infrapatellar fat pad derived expanded MSCs suspended in 1 ml of medium was delivered to the injured knee by direct intraarticular injection. Control group received 1 ml of medium without cells.

Results: Gross photographs of femoral condyles in control group at 16(A) and 20 weeks(B) after surgery. Typical cartilage lesion is more evident at 20 weeks (black arrow). Gross photographs of femoral condyles in MSC-treated group at 16 (C) and 20 weeks (D). Articular cartilage exhibited a good gross appearance particularly at 20 weeks (D).

Microscopic appearance of articular cartilage at 20 weeks after surgery stained with toluidine blue (Magnification, °—40). (A) Sham group shows normal articular cartilage with intact surface. (B) MSC-treated group represents appropriate thickness, normal distribution of the cells and cartilage matrix is consistently well stained with toluidine blue. Surface layer shows very mild irregularity. (C) Control group demonstrates, structural disorganization, fever chondrocytes hypocellularity and loss of toluidine blue staining.