Stem Cell Therapy

The ORTHOPEDIC MEDICINE PRACTICE

Michael N Brown, M.D.

The orthopedic medicine approach

• In the beginning...
  – Prolotherapy practionioner
  – Autologous blood
  – Growth factors
  – Platelet rich plasma
  – Umbilical cord blood
  – Bone marrow aspirate concentration (BMAC)
  – Autologous fat graft
  – Stromal vascular fraction

• Marc Hedrick and colleagues reported on the identification of an adherent, multi-potent cell from adipose tissue in 2001

2002-2013 studies

• The systematic research showed an increasing number of published studies on this topic over time and identified:
  – 72 preclinical papers and
  – 18 clinical trials.
  – 18 clinical trials identified focusing on cartilage regeneration, none were randomized.
  – 5 were comparative,
  – 6 were case series, and
  – 7 were case reports;
  – 2 concerned the use of adipose-derived MSCs, five the use of BMC
  – 11 the use of bone marrow-derived MSCs, with preliminary interesting findings ranging from focal chondral defects to articular osteoarthritis degeneration.
The orthopedic medicine approach

• Discogenic pain:
  – Radiofrequency nucleoplasty
  – Radiofrequency annuloplasty
  – Coblation nucleoplasty
  – Laser nucleoplasty
  – DeKompresor
  – IDET
  – DiscTrode
  – Autologous blood
  – Cryoprecipitate, plasmapheresis-platelets

Lessons from the past

• Ever wondered ...
  – What happened?
  – Where did all this technology go?
  – LESSONS FROM THE PAST

A warning from Dr. Bogduk

The orthopedic medicine approach

• Orthopedic medicine practitioner:
  – Expertise in physical diagnosis
  – Expertise in injection therapy
  – Expertise in selecting treatment modalities and complex cases
  – There will be a need for expertise in cell medicine deployment
    • Fluoroscopy
    • Ultrasonography
  – Expertise in combining cellular and biologic therapy with other regenerative technologies.
Adipose SVF...?
WHAT (AND HOW) HAVE WE BEEN DOING?

Michael N. Brown, MD

GLP, cGMP, and Standard Operating Procedures

• STEPS IN SVF:
  – Washing
  – Enzymatic digestion
  – Disruption
  – Centrifuge separation for isolation of SVF
  – Cryopreservation or Culture and Expansion

• Most academic research laboratories do not produce adipose stem cells in accordance with the criteria for either Good Laboratory Practices (GLP) or the more stringent current Good Manufacturing Practices (cGMP). Both GLP and cGMP require strict operational and certification records relating to all laboratory equipments used in the cell manufacture process.

Closed System Manufacturing Devices

• Several companies have developed self-contained lipoaspirate processing devices that collect, wash, digest, and separate cells without exposing them to the environment. Cytori...
Orthopedic stem cell science

Adipose tissue

- Subcutaneous adipose depots are ubiquitous and easily accessible in large quantities with a minimal invasive procedure (by liposuction aspiration).
- Liposuction surgery is a well-tolerated and safe procedure yielding large quantities of aspirate.
- It has been described that stem and progenitor cells in the uncultured stroma-vascular fraction (SVF) from adipose tissue usually amount to up to 3% of the whole cells, and this is 300-500-fold more than the frequency of stem cells in bone marrow.


Adipose tissue as a source of stem cell

- Adipose tissue is capable of rapid expansion secondary to its ability to proliferate the vascular supply. (1, 2)
- Stem cells surround the vessels to support neoangiogenesis.
- Stem cell counts can vary based on extraction technique:
  - Wet
  - Dry
  - Depending on tumescent

Adipose stem cell

- ASCs can easily be isolated by tissue digestion and centrifugation steps, followed by the outgrowth of the plastic adherent fraction from the primary isolated cell mixture (the so-called SVF).  
- Discrepancies in the results of studies from different laboratories may result from many different origins. First of all, ASCs are isolated from different donors. These donors differ in age, body mass index, gender, ethnicity, and their medical history (e.g., preexisting diseases, nicotine, or alcohol abuse in humans). It has been shown, for example, that the body mass index correlates negatively to the number of stromal cells per gram and their differentiation capacity.


Homing to injured tissues

- ASCs home to injured tissue.
- Systemic or regional injection of expanded ASCs after tissue injury or disease in animal models indicates cells migrate or “home” toward injured tissue.


MSC stem cells & The Meniscus

Michael N. Brown, MD
Meniscus injuries

- The vascular supply is an important factor to determine the potential healing of meniscal tears


Meniscus tears

- As the fibrocartilaginous tissue of the meniscus presents a limited regenerative capacity, new approaches are required to improve meniscal healing.
- In the last few decades, several emerging strategies, including growth factors, gene therapy, and application of mesenchymal stem cells (MSCs), have been proposed to increase healing of a damaged meniscus by tissue-engineered constructs.
- Tissue engineering is based on a combination of cells, growth factors, and scaffolds able to stimulate the meniscal healing

Peretti et al. [1] described a porcine chondrocyte model where implantation of such cells was performed in the avascular part of the meniscus, using an allogenic scaffold seeded with autologous chondrocytes, showing that these chondrocytes were able to heal a meniscal tear.
- Many methods of implant technologies have been utilized... “the holy grail of the orthopedics”

Meniscus tears

- Another potential cell therapy approach is represented by MSCs.
  - These pluripotent cells are able to differentiate into specific therapeutic cell types (developmental plasticity).\(^1\)\(^2\)
- A cell/scaffold combination, are a promising alternative for repairing large meniscal defects.\(^3\)
- Fat & Stroma as a scaffold...
  - Emulsified fat injected behind ligaments


Meniscus tears

- Several studies confirm production of abundant extracellular matrix around the cells, restoring a meniscal-like tissue in the avascular zone.\(^1\)\(^4\)
- In particular, the combination of growth factors and mesenchymal stem cells within scaffold implants increased proteoglycan and/or collagen synthesis.\(^2\)\(^5\)


Fat grafting technique

- Fat graft:
  - You are best to use 1cc syringe and use multiple syringes for control of pressure and delivery.
  - Problems can occur with fat flow – can build up pressure and flush in
  - Cytori:
    - Has developed cellbrush syringes that provide a thumb advance to smooth out the cell delivery during fat grafting procedures.
Effect of growth factors

• Transforming growth factor-β1 (TGF-β1)
• Fibroblast growth factor-2 (FGF-2)
• Basic fibroblast growth factor (bFGF)
• Insulin growth factors (IGFs), particularly IGF-1, are considered the main anabolic growth factor of articular cartilage.
• Vascular endothelial growth factor (VEGF) may promote better healing.

Meniscus tears

• Recently, platelet-rich plasma (PRP) may be better than the use of isolated growth factors.
• PRP is an autologous substance rich in platelets. It is easily prepared by spinning autologous blood in a centrifuge to form a dense fibrin matrix that can be placed directly at the meniscal repair site.

PRP augmented Rotator cuff repair

• The purpose of this systematic review was to address the treatment of rotator cuff tears by applying tissue engineering approaches to improve tendon healing, specifically platelet-rich plasma (PRP) augmentation, stem cells, and scaffolds. Our systematic search was performed using the combination of the following terms: "rotator cuff", "shoulder", "PRP", "platelet rich plasma", "scaffold", "growth factors", and "tissue engineering".
• No level I or II studies were found on the use of scaffolds and stem cells for rotator cuff repair. Three studies compared rotator cuff repair with or without PRP augmentation. All authors performed arthroscopic rotator cuff repair with different techniques of suture anchor fixation and different PRP augmentation. The three studies found no difference in clinical rating scales and functional outcomes between PRP and control groups.
• Only one study showed clinical statistically significant difference between the two groups at the 3-month followup. Any statistically significant difference in the rates of tendon rerupture between the control group and the PRP group was found using the magnetic resonance imaging. The current literature on tissue engineering application for rotator cuff repair is scanty. Comparative studies included in this review suggest that PRP augmented repair of a rotator cuff does not yield improved functional and clinical outcome compared with non-augmented repair at a medium and long-term followup.

References


Adult Human Mesenchymal Stem Cells Delivered via Intra-Articular Injection to the Knee Following Partial Medial Meniscectomy: A Randomized, Double-Blind, Controlled Study

C. Thomas Vangsness, Jr., MD1, Jack Farr, II, MD2, Joel Boyd, MD3, David T. Dellaero, MD4, C. Randal Mills, PhD5, Michelle LeRoux-Williams, PhD5

• Background: There are limited treatment options for tissue restoration and the prevention of degenerative changes in the knee. Stem cells have been a focus of intense preclinical research into tissue regeneration but limited clinical investigation. In a randomized, double-blind, controlled study, the safety of the intra-articular injection of human mesenchymal stem cells into the knee, the ability of mesenchymal stem cells to promote meniscus regeneration following partial meniscectomy, and the effects of mesenchymal stem cells on osteoarthritic changes in the knee were investigated.
• Methods: A total of fifty-five patients at seven institutions underwent a partial medial meniscectomy. A single superolateral knee injection was given within seven to ten days after the meniscectomy. Patients were randomized to one of three treatment groups: Group A, in which patients received an injection of 50 × 10^6 allogeneic mesenchymal stem cells; Group B, 150 × 10^6 allogeneic mesenchymal stem cells; and the control group, a sodium hyaluronate (hyaluronic acid/hyaluronan) vehicle control. Patients were followed to evaluate safety, meniscus regeneration, the overall condition of the knee joint, and clinical outcomes at intervals through two years. Evaluations included sequential magnetic resonance imaging (MRI).
• Results: No ectopic tissue formation or clinically important safety issues were identified. There was significantly increased meniscal volume (defined a priori as a 15% threshold) determined by quantitative MRI in 24% of patients in Group A and 6% in Group B at twelve months post-meniscectomy (p = 0.022). No patients in the control group met the 15% threshold for increased meniscal volume. Patients with osteoarthritic changes who received mesenchymal stem cells experienced a significant reduction in pain compared with those who received the control, on the basis of visual analog scale assessments.
• Conclusions: There was evidence of meniscus regeneration and improvement in knee pain following treatment with allogeneic human mesenchymal stem cells. These results support the study of human mesenchymal stem cells for the apparent knee-tissue regeneration and protective effects.


Many recipies and techniques

• Added post-derived stem cell isolation techniques. The wide variance in that time, materials, and effortful obtaining ASCs via standard isolation and/or rapid isolation techniques.
• A highly viable population of around 250,000 ASCs can be derived from 250 cc of blood/saline fraction of the liposuction waist in as little as 30 minutes, compared to 8-10 hours to obtain ASCs using the traditional isolation method.
• SVF(stromal vascular fraction), ABAM, antibiti / antimycotic ; FBS, fetal bovine serum.

Stem cell for lumbar degenerative disc disorder
The lumbar disc

Intervertebral Disc Cell Therapy for Regeneration:
Mesenchymal Stem Cell Implantation in Rat Intervertebral Discs

- This study explores the use of mesenchymal stem cells (MSCs) for intervertebral disc regeneration.
- We used an in vivo model to investigate the feasibility of exogenous cell delivery, retention, and survival in the pressurized disc space.
- MSC injection into rat coccygeal discs was performed using 15% hyaluronan gel as a carrier. Injections of gel with or without MSCs were performed. Immediately after injection, fluorescently labeled stem cells were visible on sections of cell-injected discs.
- 7-14 days after injection, stem cells were still present within the disc, but their numbers were significantly decreased. At 28 days, a return to the initial number of injected cells was observed, and viability was 100%.
- A trend of increased disc height compared to blank gel suggests an increase in matrix synthesis. The results indicate that MSCs can maintain viability and proliferate within the rat intervertebral disc.


Feasibility of a stem cell therapy for intervertebral disc degeneration

- In vivo studies of healthy adult rabbit discs injected with allogenic adult rabbit MSCs to examine stem cell survival and engraftment in living disc tissue.
- MSCs were isolated from the bone marrow of a New Zealand White (NZW) rabbit
- MSCs were detected in histological sections of rabbit discs up to 24 weeks after allogenic stem cell implantation,
- The 24-week results in particular suggested the possibility of stem cell migration and engraftment into the inner annulus fibrosus.
- transplanted MSCs survive and successfully engraft into the IVD tissue

Satoshi Sobajima, et al., The Spine Journal Volume 8, Issue 6, Pages 888-896, November 2008
Patient selection in disc cases...

- How do we select cases?
- Do we want to hydrate a disc?
- You can make things worse!
- Complex physiology with chondrocytes in DDD.
  - We know now that we could...
  - But now we need to ask if we should?
- We are going to have to categorize the patients disc syndrome and intradiscal pathomechanics to determine what method will be used...

Epidural abscess and cauda equina syndrome after percutaneous intradiscal therapy in degenerative lumbar disc disease.

- Describes a significant complication of a percutaneous intradiscal bone marrow and adipose tissue transplantation for symptomatic lumbar disc degeneration
- METHODS:
  - Two weeks after an injection of adipose cells, bone marrow aspirate and plasma into his L3-L4 and L5-S1 lumbar discs, a 64-year-old patient presented to the emergency room with cauda equina syndrome, fever, and back pain. Magnetic resonance imaging diagnosed L3-L4 disc extrusion, discitis with osteomyelitis, and epidural abscess, resulting in emergency decompressive surgery. An epidural abscess was drained, extruded disc material was removed, and cultures obtained. Five days later, once afebrile on antibiotics, he underwent a definitive interbody arthrodesis and stabilization.


Epidural abscess and cauda equina syndrome after percutaneous intradiscal therapy in degenerative lumbar disc disease.

- RESULTS:
  - Cauda equina syndrome resolved, osteomyelitis (methicillin-resistant Staphylococcus epidermidis) was treated, and instrumented arthrodesis stabilized the involved segment.
- CONCLUSIONS:
  - Complications associated with the intradiscal injection of agents, such as stem cells, fibrin glue, adipose tissue, or bone marrow, have been poorly defined. Given the nature of the degenerating disc, serious adverse events, including discitis, osteomyelitis, and extrusion of disc contents, may occur.

Transplantation of Human Adipose-Derived Stem Cells in a Rabbit Model of Traumatic Degeneration of Lumbar Discs

METHODS: 20 mature male New Zealand white rabbits. Intervertebral discs were injured in each rabbit by percutaneous technique at L2-3, L3-4, and L4-5 under C-arm guidance with a 19-gauge spinal needle. Magnetic resonance images (MRI) were checked at 6, 9, 12, and 15 weeks after injury to evaluate disc degeneration.

Nineteen weeks after injury, ADSCs from human fat was injected into the L4-5 disc space, with saline injected into the L3-4 disc as a control, using a 21-gauge spinal needle. Histologic confirmations of degenerated discs were performed at 10 and 18 weeks after injury with safranin O and trichrome stains.

RESULTS: MRI revealed intervertebral disc degeneration from 9 weeks after injury, and full degeneration at 15 weeks after injury, when compared with uninjured control discs. We confirmed the proliferation of ADSCs at the L4-5 level in 10-week rabbits after cell injection. Histologically, the ADSC-injected discs exhibited elevated extracellular matrix secretion and little ossification of damaged cartilage in the nucleus pulposus compared with degenerative control discs.

CONCLUSIONS: These results suggest that the injection of ADSCs into injured lumbar discs could be an effective treatment for degenerative disc disease by promoting the cartilage regeneration.

Hyoung-Joon Chun, Young Soo Kim

Intradiscal ADCs

- Histologic findings at 29 weeks after injury (32 weeks after ADSC injection); slides (A and B) with degenerative intervertebral disc by percutaneous technique has several histologic findings, including destruction of lamellar pattern, decreased cellularity, deviated extracellular matrix, and fibrocartilaginous change. However, the histologic features of the intervertebral disc injected with ADSC (C and D) shows reconstruction of lamellar pattern and increased cellularity. (A and C, safranin O staining; B and D, trichrome staining. High-power microscopy [X100].)

Intradiscal ADCs

- Histologic slide from 37 weeks after injury (38 weeks after ADSC injection). When compared with

  - the histologic features of 29 weeks after injury (degenerative changes were more aggravated in A and B, and

  - recovery patterns were more clear in C and D. High-power microscopy [X100].)
BMAC intradiscal injection

- Cells isolated from bone marrow (but with no characterisation of the cells) were injected into human discs of individuals with discogenic pain [1].
- This treatment was followed by 2 weeks of hyperbaric oxygen therapy, nominally to improve the oxygen supply to the implanted cells.
- Twelve months following treatment there was no relief in back pain in the treated patients and 75% progressed to a spinal fusion indicating that these are not likely to be an appropriate source of cells.


Tissue engineering and the intervertebral disc: the challenges

- Areas that require development before the technique could be utilized.

Comparative Study on Functional Effects of Allotransplantation of Bone Marrow Stromal Cells and Adipose Derived Stromal Vascular Fraction on Tendon Repair: A Biomechanical Study in Rabbits

- compared effects of allotransplantation of bone marrow stromal cells (BMSCs) and adipose derived stromal vascular fraction (SVF) on tendon mechanical properties after experimentally induced flexor tendon transection.

- Results:
  - The results of this study showed stromal cell transplantation resulted in significant increase in ultimate and yield loads, energy absorption, and stress of repairs compared to the controls. However, statistically significant changes were not detected in terms of stiffness. In comparison, no significant differences were found at third week between SVF and BMSCs treated tendons in terms of all load related properties.
  - However, at the eighth week SVF transplantation resulted significantly increased in energy absorption, stress and stiffness compared to BMSCs.

Comparison Study on Functional Effects of Allotransplantation of Bone Marrow Stromal Cells and Adipose Derived Stromal Vascular Fraction on Tendon Repair: A Biomechanical Study in Rabbits.

**Conclusion:**

- The enhanced biomechanical properties of repairs in this study advocates the application of adipose derived SVF as an excellent source of **multipotent cells** instead of traditional BMSCs and may seem more encouraging in cell-based therapy for tendon injuries.

**Favors SVF...**

**ASC in nerve regeneration**

- Another emerging technology for the future is the use of adipose derived stem cells (ASC). These cells derived from autologous fat via lipoaspiration have features of multipotential differentiation.
- These cells which can be harvested and digested from the fat stroma and concentrated has been shown to have transcripts for nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF) and neurotrophin-3 (NT3) which has been detected in both deep and superficial fat layers in the abdomen.
- ASC shown three times more nestin-positive cells in culture taken from fat than from bone marrow.
- Nestin may help ASC differentiate into neuro-glial lineage and thus ASC may be used for repair of other structures than just mesodermal lineage.

**ASC in nerve regeneration**

ASC in nerve regeneration

- Interestingly cells cultured from lipoaspirates in the more superficial layers of the fat over abdomen were found to be a better source of ASC to use in nerve repair.1
- These cells from the superficial layer significantly enhance neurite outgrowth in culture compared to the deep layers and did not require stimulation to elicit this response in study with rat cells.
- ASC isolated from the superficial layer of fat significantly enhanced neural outgrowth compared deeper layers.2

ADC immunomodulation

- In vitro, ASCs inhibit proliferation of activated lymphocytes through both cell-cell binding and paracrine signaling.
- More important, T-cell suppression is not impaired by the loss of cell-cell contact of lymphocytes and ASCs.
- In vivo, expanded ASCs have demonstrated immunosuppressive properties in mice, alleviating graft-versus-host disease (GVHD), colitis and arthritis.

Mechanisms ASCs use to suppress immunity

- ASCs are positive for Thy-1 expression, an inflammation- Q8 dependent adhesion molecule that mediates interactions with monocytes, granulocytes, and melanoma cells.1
- During oxidative stress, ASCs secrete transforming growth factor β1, a growth factor known to promote premature helper T-cell differentiation toward T regulatory cells, which promote immune tolerance.2
- ASCs also secrete galectin-1 and -3, which have been shown to be essential paracrine molecules in T-cell suppression. Last, ASCs metabolize L-arginine, limiting the bioavailability of a crucial molecule for T-cell proliferation and function.3

ADC immunomodulation

- The authors concluded that the ASC-mediated therapeutic effect was associated with a down regulation of the T helper 1-driven inflammatory response.
- Furthermore, measurement of a wide panel of inflammatory cytokines and chemokines in blood showed inflammatory cytokines were decreased and IL-10 was increased, potentially enhancing T regulatory cell numbers.

ADC immunomodulation

- Multiple sclerosis is an autoimmune disorder affecting white matter of the brain and the spinal cord resulting from a loss of oligodendrocytes and a thinning or complete loss of myelin.
- A series of case studies using intravenous infusions of 25–75 million SVF cells reported improved cognition and an almost complete reduction of extremity spasticity.

That’s 50 – 75 million cells folks!


Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis.

- PURPOSE: In the present study, the clinical outcomes and second-look arthroscopic findings of intra-articular injection of stem cells with arthroscopic lavage for treatment of elderly patients with knee osteoarthritis (OA) were evaluated.
- METHODS: Stem cell injections combined with arthroscopic lavage were administered to 30 elderly patients (≥65 years) with knee OA. Subcutaneous adipose tissue was harvested from both buttocks by liposuction. After internal vascular fractions were isolated, a mean of 4.04 × 10^6 stem cells (9.7% of 4.16 × 10^7 stromal vascular fraction cells) were prepared and injected in the selected knees of patients after arthroscopic lavage. Outcome measures included the Knee Injury and Osteoarthritis Outcome Scores, visual analog scale, and Lysholm score at preoperative and 1-, 2-, and 3-year follow-up visits. Sixteen patients underwent second-look arthroscopy.
- RESULTS: Almost all patients showed significant improvement in all clinical outcomes at the final follow-up examination. All secondary outcomes and radiographs at 2-year follow-up (P < 0.01). Among elderly patients aged >65 years, only five patients demonstrated worsening of Kellgren-Lawrence grade. On second-look arthroscopy, 87.5% of elderly patients (14/16) improved or maintained cartilage status at least 2 years postoperatively. Moreover, none of the patients underwent total knee arthroplasty during this 2-year period.
- CONCLUSION: Adipose-derived stem cell therapy for elderly patients with knee OA was effective in cartilage healing, reducing pain, and improving function. Therefore, adipose-derived stem cell treatment appears to be a good option for OA treatment in elderly patients.

Koh YG1, Choi YJ, Kwon SK, Kim YS, Yeo JE. Knee Surg Sports Traumatol Arthrosc. 2013 Dec 11
A novel biological approach to treat chondromalacia patellae

The stem-cell-containing stromal vascular fraction was mixed with calcium chloride-activated platelet-rich plasma and hyaluronic acid, and this ADSCs mixture was then injected under ultrasonic guidance into the retro-patellar joints of all three patients.

Patients were subjected to pre- and post-treatment magnetic resonance imaging (MRI) scans. Pre- and post-treatment subjective pain scores and physical therapy assessments measured clinical changes.

One month after the injection of autologous ADSCs, each patient’s pain improved 50-70%. Three months after the treatment, the patients’ pain improved 80-90%. The pain improvement persisted over 1 year, confirmed by telephone follow ups.

Also, all three patients did not report any serious side effects. The repeated magnetic resonance imaging scans at three months showed improvement of the damaged tissues (softened cartilages) on the patellar-femoral joints. In patients with chondromalacia patellae who have continuous anterior knee pain, percutaneous injection of autologous ADSCs may play an important role in the restoration of the damaged tissues (softened cartilages).

Thus, ADSCs treatment presents a glimpse of a new promising, effective, safe, and non-surgical method of treatment for chondromalacia patellae.
• Figure 4. MRI axial sequential T2 views patient 3. Pre-treatment MRI scans of patient 3 (A [4/19], C [5/19], and E [6/19]) show retropatellar signal changes (arrow) consistent with chondromalacia patellae along with medial meniscal maceration and cartilage thinning consistent with osteoarthritis. Post-treatment MRI scans at three months (B [5/20], D [6/20], and F [7/20]) show changes (arrowhead) consistent with probable cartilage restoration.

Improvement with ADC in patellofemoral disease

• Pain measurements of patients 1 (A), 2 (B), and 3 (C).
• VAS is visual analog scale and T bars indicate standard deviations.

Comparison between stem cells harvested from wet and dry liposomates

• Adipose-derived stem cells (ASC) are usually isolated from liposomates, but it is not known if the anesthetic solution injected into adipose tissue affects cell yield and functions. Two different samples were drawn from the abdominal region of female subjects. In the first, a physiological solution containing lidocaine/adrenaline was injected (wet liposuction, WL), while in the contralateral area, the sample was collected without injecting any solution (dry liposuction, DL). The aspirates were processed to investigate the yield of the stromal-vascular fraction (SVF) cells and ASC frequency, growth rate, apoptosis, and differentiation potential.

The solid dried mass of fresh WL isolates was lower than that of DL isolates (<p> 0.01) due to the presence, in the former, of a liquid solution. As a consequence, the amount of WL-SVF cells was 18.7% lower than those obtained from DL (<p> 0.01); this difference was also observed under culture conditions. In addition, the number of colony-forming unit-fibroblasts (CFU-Fs) obtained from 1 × 10³ SVF cells was 25.5% lower in WL-aspirates than DL-aspirates (<p> 0.05) owing, at least in part, to the observed presence of ASC (corrected) in the liquid solution of the WL isolates. After WL and DL, no differences were observed in ASC growth rate, apoptosis, or differentiation potential toward adipogenic, osteogenic, and endothelial cell lineages. In conclusion, WL yields about 40% fewer ASC than DL due to the combined effect of tissue dilution and the reduced frequency of ASC in the SVF. The main biological features of ASC are suitable for cell-based therapies.
Use of MSCs in OA

- Whatever the source of MSCs (BM, adipose tissue or synovium), factors secreted by MSCs increased cartilage matrix production by chondrocytes.
- Mechanism is not known. Possible paracrine effect...
- ASCs were shown to protect OA chondrocytes against apoptosis and degeneration (1).

1. Marie Maumus, Christian Jorgensen, Danièle Noël. Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: Role of secretome and exosomes Biochimie 95 (2013) 2229-2234

Use of MSCs in OA

- Although OA is not considered an inflammatory disease, pro-inflammatory mediators, such as cytokines, metalloproteinases (MMP), reactive oxygen species (ROS), are secreted by OA chondrocytes or synoviocytes and, participate to joint tissue alterations.
- Several pro-inflammatory cytokines are significantly downregulated in chondrocytes when cultured with ASCs suggesting that ASCs may also be protective through the down-regulation of inflammatory mediators (1).
- Interestingly, paracrine factors of BM-MSCs share the same anti-inflammatory effects on OA cartilage and synovial explants in vitro (2).


Use of MSCs in OA

- Local injection of BM-MSCs or ASCs in the joint is likely to exert several roles: inhibition of osteophyte formation, decrease of synovial inflammation, reduction of cartilage degeneration with less fibrosis and apoptosis of chondrocytes or stimulation of chondrocyte proliferation and extracellular matrix synthesis (Fig. 2). Indeed, using the pre-clinical murine model of collagenase-induced OA, a single injection in the knee joint of mice inhibited synovial activation and formation of chondrophyte/osteophyte in joint ligaments as well as cartilage destruction, probably by suppressing synovial macrophage activation (1).

Effects of MSCs

Use of MSCs in OA
Protective and regenerative

- Intra-articular injection of BM-MSCs can also prevent the development of post-traumatic arthritis [1].
- Other preclinical studies using larger animal models of OA (rat, rabbit, guinea pig, sheep, donkey and goat) revealed similar results with cartilage regeneration after injection of MSCs in the damaged joint [2-7].
- Intra-articular injection of autologous BM-MSCs in six patients with knee OA was safe and improved pain, functional status of the knee. MRI displayed increased cartilage thickness and decrease of subchondral edema in 3 of 6 patients [8].
References


Whatever the source of MSCs (BM, adipose tissue or synovium), factors secreted by MSCs increased cartilage matrix production by chondrocytes [25].

Stem Cell Therapy in a Caprine Model of Osteoarthritis

• Adult stem cells were isolated from caprine bone marrow, expanded in culture, and transduced to express green fluorescent protein.
• 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 cells suspended in a dilute solution of sodium hyaluronan was delivered to the injured knee by direct intraarticular injection.
• 10 nM dexamethasone, 10 nM -glycerophosphate, 50 M ascorbic acid 2-phosphate, 100 nM prostaglandin E2,
• Dexamethazone...


Scutt A, Bertram P. Basic fibroblast growth factor in the presence of dexamethasone stimulates colony formation, expansion, and osteoblastic differentiation by rat bone marrow stromal cells. Calcif Tissue Int 1999; 64:69-77.


Stem Cell Therapy in a Caprine Model of Osteoarthritis

• No immobilization or splinting of the joints was used and animals were bearing weight on the operated joint within 3–5 days.

• Appearance of meniscal-like repair tissue, or neomeniscus, was observed in association with the posterior medial compartment of cell treated knees at 6 weeks after injection tissue was hyaline in nature and appeared to provide a bearing surface for the tibial and femoral condyles.

J. Mary Murphy, David J. Fink, Ernst B. Hunziker, and Frank P. Barry

Stem Cell Therapy in a Caprine Model of Osteoarthritis

• There was also evidence of vascularization in the neomeniscus close to the point of synovial attachment.

• Twenty weeks after delivery of cells there was again evidence of repair tissue associated with the posterior medial compartment in 7 of 9 treated joints.

• Functional entheses with the tibial bone were formed with repair of the meniscal insertional ligaments

• Areas of articular cartilage where there was improvement and others where there were not.

J. Mary Murphy, David J. Fink, Ernst B. Hunziker, and Frank P. Barry

ARTHRITIS & RHEUMATISM Vol. 48, No. 12, December 2003, pp 3464–3474
Stem Cell Therapy in a Caprine Model of Osteoarthritis

• MSCs injected into the knee joint did not bind to normal or fibrillated articular cartilage in vivo, but we cannot exclude the hypothesis that the cells may act to mediate OA progression by an effect other than that seen on meniscal regeneration.

• This is a scaffold-free method for cell delivery and is therefore unencumbered by the complexities associated with placement of a solid cell construct.

• Other options:
  – direct implantation of meniscal cells, chondrocytes, or stem cells, perhaps in combination with delivery of appropriate mitogens or growth factors to stimulate host cell proliferation, may lead to a sustained therapeutic effect.

SVF isolation technology

• It is likely that adoption of a dominant technology will follow whichever device first receives Food and Drug Administration approval, which will allow for some industry-wide standardization in the stem cell isolation process.

• A standardization of SVF isolation will allow for easier comparison of therapy efficacy across studies and will increase technique use in established tissue regenerative applications.

Multilineage potential

• Multilineage potential. ASCs have demonstrated a broad in vitro capacity for differentiating toward cell types of all 3 germ layers (Table III).6,29-32

• Clinically, this suggests that ASCs may have wide utility in a range of regenerative strategies for multiple tissue types.

• Undifferentiated ASCs have been seeded on a variety of natural and polymer scaffolds, and have been examined in vivo for bone,33-35 cartilage,36 adipose,37 and skin38 regeneration.
## Automated stromal vascular fraction isolation devices

<table>
<thead>
<tr>
<th>Device</th>
<th>Manufacturer</th>
<th>Clinical use</th>
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<tbody>
<tr>
<td>Celution</td>
<td>Cytori Therapeutics Inc, San Diego, CA</td>
<td>Cell-enriched stromal vascular fraction, chronic ulcer, complex cryptoglandular fistula-in-ano, urinary incontinence, systemic sclerosis, myocardial ischemia, osteopenic fractures</td>
</tr>
<tr>
<td>Tissue Genesis Isolation System (Icellator)</td>
<td>Tissue Genesis, Honolulu, HI</td>
<td>Cell-enriched stromal vascular fraction, peripheral vascular disease</td>
</tr>
<tr>
<td>Lipid fill</td>
<td>Medi-Kahn</td>
<td>Cell-enriched stromal vascular fraction, extremely ulcer</td>
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<td>CHA-STATION, Multistation</td>
<td>Advanced Bio Medical Equipment Co</td>
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### The SVF Secretome

The figure depicts a Venn diagram demonstrating proteins that are expressed in the secretome of the 5 major studies referred to in this review (Kim et al., Zhong et al., Zvonic et al., Mutch et al., Rosenow et al.). In all these studies ASCs or SVF cells or preadipocytes were allowed to differentiate toward adipocytes. The conditioned media was analyzed to detect secreted proteins. The overlapping regions of the sets represent the number of secretome proteins that are in common among the studies.

### The SVF Secretome

Upregulated and downregulated proteins. This figure depicts a Venn diagram demonstrating the proteins that are upregulated and downregulated in the secretome. The overlapping regions of the sets represent the number of proteins that are in common among the studies (Kim et al., Zhong et al., Mutch et al.).
Clinical effects of the ASC secretome

- Adipose derived stem cells have been shown to exercise their beneficial effects through differentiation and via paracrine mechanisms.
- The importance of this paracrine effect may be more important than previously perceived. (1)

Angiogenesis and revascularization

- Proangiogenic properties of the ASC secretome have been demonstrated using rodent limb ischemia and acute myocardial infarction models.
- Rehman et al. compared the angiogenic effects of injected ASCs to ASC conditioned media in a rodent hindlimb ischemia model.
  - They found that cultured SVF stimulated neoangiogenesis.
  - On analysis of the conditioned media granulocyte colony stimulating factor (G-CSF), transforming growth factor beta (TGF-b), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF) were detected. (1)

Immunomodulation

- ASCs were found to have a greater anti-inflammatory effect than BM-MSCs on in vitro preparations of monocyte derived dendritic cells. This was seen in the greater reduction of dendritic cell surface markers (CD80, CD83, CD86) and higher up-regulation of the anti-inflammatory cytokine, interleukin-10 (IL-10), by ASCs. (1)
- ASC infusion in a murine experimental colitis model led to a decrease in the level of pro-inflammatory cytokines (TNF-a, IFN-g, IL-6, IL-10 and IL-12) and an increase in level of anti-inflammatory IL-10 and IL-10 secreting regulatory T cells. (2)
MSCs in RA

- Focusing on rheumatic diseases, it is likely that the route of MSC administration will differ according to the pathology.
- In case of systemic disease, such as rheumatoid arthritis (RA) where several joints may be affected, systemic delivery via the bloodstream should be favored. Local injection + IV
- On the contrary, for lesions that are limited to a single joint, local delivery should be preferred because of better availability of cells and safety.

References

1. Marie Maumu, Christian Jorgensen, Daniele Noél, Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: Role of secretome and exosomes Biochimie 95 (2013) 2229-2234

Conflicting evidence in RA

- Besides reduced levels of pro-inflammatory cytokines in mouse sera, mechanisms involved in reduction of clinical signs were suggested to be through CD4, CD25, Foxp3, Treg cell induction as well as T-cell anergy [2-5].
- In contrast, other studies failed to demonstrate any improvement with MSC treatment. Systemic infusion of the allogeneic C3H10T1/2 cell line did not decrease the clinical signs of arthritis [7].
- Similar results were obtained using primary murine MSCs isolated from different strains of mice suggesting that different genetic backgrounds influence the immunosuppressive effect of BM-MSCs [8].
Improved wound healing

- Improved wound healing. (1, 2)
- Microarray analyses have demonstrated that ASC spheroids upregulate multiple extracellular matrix (ECM) genes (Insulin derived growth factor-1 (IGF-1), insulin derived growth factor binding protein 5 (IGFBP-5), periostin, transforming growth factor beta-1 (TGF-β1), transforming growth factor beta-2 (TGF-β2), Syndecan 1, Syndecan 2, Biglycan, Collagen VIII A2, Collagen XIV A2, Collagen XV A2, Collagen XVIII A1, Collagen VI A3, Decorin, Elastin, Fibronectin 1, Laminin A1, Laminin B1, MMP-1,2,3,14, TIMP-1,2,3 and Tenascin C) along with many growth factors. (3)


Significant differences between BM-MSCs and ASCs have been reported. The cytokine profile of ASCs and BM-MSCs differs. (1)


MSCs in neuro cases

- ASCs also represent a powerful tool for neurodegenerative medicine. ASCs can stimulate the regeneration of nervous tissues by promoting nerve healing and de novo axon growth, via the release of several neurotrophic factors such as brain derived neurotrophic factor (BDNF), nerve growth factor (NGF) or glial derived neurotrophic factor (GDNF). (1)
  - They protect neurons against apoptosis (2)
  - Slows disease progression in models of Huntington disease (3)

RISKS of stem cell

- Breitbach et al. described calcification of MSCs injected into infarcted rat hearts.
- For the treatment of articular cartilage defects, this implies in theory the risk of such MSC-mediated endochondral ossification to occur at least in some parts of the repair tissue, thus jeopardizing the formation of good-quality tissue and the clinical outcome.


Thank you