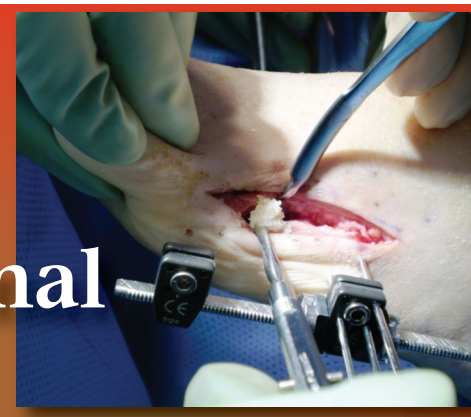
New Ideas in Clinical Podiatry

Continuing Medical Education

The Use of Adult Mesenchymal Stem Cells in Reconstructive Foot Surgery



Recent research shows great promise for the use of these cells.

Objectives

1) To know the differences between adult mesenchymal stem cells and embryonic stem cells.

2) To understand how adult mesenchymal stem cells are distinguished and how they are activated to form various types of tissue.

3) To be able to understand all the clinical applications of adult mesenchymal stem cells.

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*Following this article, an answer sheet and full set of instructions are provided (p. 218).—**Editor***

By Howard Kimmel, DPM

Stem cells by definition are primal undifferentiated cells that can differentiate into multiple cell types. The body has a reservoir of these cells in various tissues. Adult stem cell therapy is an evolving exciting therapy in medicine. Adult stems cells play a significant role in the daily maintenance of the body. These adult mesenchymal stem cells are completely different than the controversial embryonic stem cell. Embryonic stem cells result from the egg and sperm at conception. They are totipotential, which means that they have the capacity to grow into any cell type without exception. The adult stem cell has limited potential for differentiation compared to the embryonic stem cell.

Adult mesenchymal stem cells can differentiate into one, two, or many cell types. In essence, the adult mesenchymal stem cell is multipotent. They can differentiate into various types of tissues including, but not limited to bone, cartilage, adipose, and endothelium cells. As the embryonic stem cells develop into adult stem cells, they lose their totipotential ability, but increase their specialization. In 1924, Alexan-

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der Maxinow used histological findings to describe a single precursor cell within the mesenchymal cell that differentiates into various types of blood cells.[1]

Anatomy and Embryology

When the embryo forms, the mesoderm, which is the middle cell layer, eventually differentiates into bone, cartilage, fat, dermis, or other connective tissue.[2] This developmental process is relatively rapid. The original embryological genesis of discrete morphologies requires a unique series of signaling events that most likely function during the embryonic process which is called the mesengenic process. This process occurs in steps in which there are cellular transitions from mesenchymal stem cells to highly differentiated phenotypes. This process continues throughout maturation and adulthood but at a significantly slower pace than in the embryo, but continues throughout life to ensure that once tissue gets injured, it is repaired. There are other signals that control the maintenance and repair involved in regenerating existing structures.[3]

The mesenchymal stem cells at a repair site mitotically expand and form a repair blastema that spans the break of tissue discontinuity; this blastema eventually differentiates into the appropriate skeletal tissues as determined by the local concentration of surrounding tissue-specific cytokines and growth factors.[4] The reason why there is limited tissue repair in the adult compared to the embryo is that there are too few mesenchymal stem cells in the adult.[5]

Repair of Injured Tissue

The process of repair of injured tissue or bone is very similar. Mesenchymal stem cells are situated throughout the body as pericytes. With the disruption of blood vessels in or near the injured tissue, stem cells are liberated from the pericytes; these cells divide and secrete bio-active factors that function to protect and repair or regenerate the injured tissue. The secretory products of mesenchymal stem cells at sites of injury strongly repress the immune response and inhibit the T and B cell-mediated destruction of the injury site.[6]

First, mesenchymal stem cells at the repair site proliferate to generate a substantial number of new mesenchymal stem cells. Various factors control this process. The mesenchymal stem cells at the repair site are then committed and enter a specific lineage pathway, i.e., bone. For a mesenchymal stem cell to turn into bone, a bone morphogenetic protein signals this differentiation. Once these cells are committed along this pathway, they continue to undergo these distinctive changes. This process is called linear progression.

End-Stage Differentiation

As these cells continue to go along this pathway they are able to divide and expand their numbers.

For a mesenchymal stem cell to turn into bone, a bone morphogenetic protein signals this differentiation.

They then undergo a terminal differentiation into their final form. This end-stage differentiation produces a cell that has a specific half-life that will eventually expire.[7]

This end-stage differentiation occurs when cell division stops. There are no unique markers known for mesenchymal stem cells, but their cell surface antigen profile has been investigated, and a comprehensive list of surface markers have been documented. Mesenchymal stem cells, by definition, must be positive for the following surface markers SH2, SH3, CD29, CD44, CD71, CD73, CD90, CD105, CD106, CD120, CD124, and CD166 while negative for CD45, CD 34, and CD14.

Hematopoietic Stem Cells

Hematopoietic stem cells are positive and exclusive for CD 34 and CD 45. Other identifying characteristics of osteogenic mesenchymal stem cells include biochemical indicators such as alkaline phosphatase, osteocalcin, and the production of a calcium rich mineralized extracellular matrix.[8]

Studies have shown that injected mesenchymal stem cells have hastened hematopoietic recovery after bone marrow transplantation, and their immune-regulatory properties facilitate engraftment of transplanted organs and reduce graft-versus host disease.[9] This effect was initially described for T cells. In vitro studies demonstrated that mesenchymal stem cells were able to suppress T lymphocyte activation and proliferation in response to alloantigens and nonspecific mitogens.[10]

Allogenic Mesenchymal Stem Cells

Allogenic mesenchymal stem cells do not express class II antigens, and therefore do not provoke an adverse host response. Class II surface antigens are required for a host to mount a T-Cell reaction to foreign cells. Two important products that stem cells produce are bone morphogenetic proteins which are needed for osteo-induction and cytokines that modulate an anti-inflammatory and immune response.

Bone marrow has two types of stem cells, those that are of hematopoietic origin, and ones derived from the mesoderm. Mesenchymal stem cells are small in number compared to hematopoietic ones. They only represent about .01% of nucleated bone cells.[11] The highest concentrations of mesenchymal stem cells are found in the pelvic girdle and the vertebral bodies. When the iliac crest is aspirated, there are roughly between one to five mesenchymal stem cells per 500,000 nucleated cells.

On average, in 1 cc. of adult marrow from an iliac crest, there will be a yield of roughly 1,000 to 1,500 mesenchymal stem cells.[12] Caplan has documented that the newborn has one mesenchymal stem cell per 10,000 marrow cells. Teenagers have one mesenchymal stem cell per 100,000 marrow cells. At age thirty, there is one per 250,000 marrow cells, and at age

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eighty, there is one mesenchymal stem cell per 2 million marrow cells.[13]

Along with age, mesenchymal stem cells are decreased with alcohol abuse, systemic illness, and osteoporosis.

Properties of Mesenchymal Stem Cells

As described above, mesenchymal stem cells have the ability to be transplanted between unmatched donor and recipient without stimulating an immune response. When dealing with the human skeleton, mesenchymal stem cells are needed for the remodeling and repair of bone and other tissue. The seminal event for all bone formation is the mesenchymal stem cell's differentiation into an osteoblast.

Once the bone morphogenetic proteins activate the mesenchymal stem cells, osteoblasts are formed to lay down new bone. These osteoblasts will either die or become osteocytes. Mesenchymal stem cells have the ability to seek out damaged tissue whether it is with a fracture, myocardial infarction, or ischemic brain injury.[14]

Clinical Applications

Currently, there is a significant amount of research regarding the use of adult mesenchymal stem cells. This research is related to both allografts and autografts. Due to the fact that mesenchymal stem cells possess anti-inflammatory and immune properties, there are current studies using the cells for graft versus host disease, along with Crohn's disease.

The low immunogenicity of these cells suggests that mesenchymal stem cells can be transplanted universally without matching between donors and recipients. In an open-label trial studying treatment of newly diagnosed graft versus host disease, 94% (29 out of 31) of available patients responded to mesenchymal stem cells with a reduction in acute (partial and complete) response. 77% of the patients had a complete response of graft versus host disease (complete resolution of disease) by day 28, indicating a durable response to treatment.

In an open-label phase-2 trial testing for treatment-resistant Crohn's disease, every patient treated had a reduction in disease severity by day 28.

At six months, 61% of the patients treated with mesenchymal stem cells still had a durable response requiring no additional immunosuppressive therapy, clinical intervention, or increased steroid use.[15] Previously published data indicates that less than 35% of patients achieve this endpoint when treated with steroids alone.

In an open-label phase-2 trial testing for treatment-resistant Crohn's disease, every patient treated had a reduction in disease severity by day 28. In patients who failed available drugs for Crohn's disease, there was a statistically significant reduction in the mean Crohn's Disease Activity Index (CDAI) score of 105 points by day 28.

The improvement was rapid with an average CDAI reduction of 62 points by day 7. There appeared to be a positive correlation between dose and response, with patients receiving the high dose achieving a greater response (average CDAI reduction of 137 vs. 65). In this difficult-to-treat population that had failed previous therapies, one-third of the patients achieved clinical remission of their disease.[16]

A study that compared human mesenchymal stem cells derived from bone marrow, periosteum, synovium, skeletal muscle, and adipose tissue revealed that synovium-derived mesenchymal stem cells exhibited the highest capacity for chondrogenesis.

Cardiomyocytes

Mesenchymal stem cells can also be induced to differentiate in vitro into cardiomyocytes, which has stimulated a large number of animal and clinical studies to evaluate the efficacy of mesenchymal stem cells for cardiac repair and regeneration.[17]

In a recent issue of the Journal of the American College of Cardiology, a group of 53 patients who had myocardial infarction were studied for two years. These patients were implanted with allograft adult mesenchymal stem cells within ten days of the event. The study was a double-blinded and randomized control. The study participants had an MRI and an echocardiogram at six months.

Patients who had received the stem cells had evidence of new blood vessel formation and a decrease in arrhythmias. The hypothesis is that adult mesenchymal stem cells have the potential to develop into mature heart cells and form new blood vessels.[18] Difficulties may arise, however, because of the broad differentiation capacity of mesenchymal stem cells. There remains significant heterogeneity among mesenchymal stem cell populations and thus they are less predictable when implanted. Most notably, some studies found that implanted mesenchymal stem cells had differentiated into osteoblasts inside ventricular tissue.[19]

Osteoarthritis

Another example in which adult mesenchymal stem cells are being investigated is its use in os-

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teoarthritis. One of the forms of tissue that mesenchymal stem cells can differentiate into is cartilage. The cells can be stimulated into chondrogenesis, and can be expanded in the laboratory.

A study that compared human mesenchymal stem cells derived from bone marrow, periosteaum, synovium, skeletal muscle, and adipose tissue revealed that synovium-derived mesenchymal stem cells exhibited the highest capacity for chondrogenesis, followed by bone marrow derived and periosteum-derived mesenchymal stem cells.[20]

Certain growth factors such as fibroblasts and transforming growth factor beta are used in an in vitro environment to grow new cells.[21] Optimization of chondrogenesis to generate stable cartilage suitable for clinical use is likely cell source-dependent, and will likely be a function of cellular context, microenvironment, as well as properties of dose and timing of the molecules administered to the cells.[22]

Mesenchymal stem cells have been transplanted in various ways into joints. In one study, autologous mesenchymal stem cells in a dilute solution of sodium hyaluronan were directly injected into the knee joints of goats, in which osteoarthritis had been induced by a total medial meniscectomy and resection of the anterior cruciate ligament. The joints that were exposed to mesenchymal stem cells showed evidence of marked regeneration of the medial meniscus, and implanted cells were detected in the newly formed tissue.[23]

Another method of application in a joint is via a scaffold. The ideal template should have the following characteristics: it needs to be biodegradable and bio-compatible along with being porous to allow the cells to penetrate and to have tissue in-growth. It should also have a surface that allows the cells to attach and migrate. There are both synthetic and natural scaffolds. Synthetic scaffolds can be designed to have all the above characteristics. They are usually made of alpha hydroxyl polyester.[24]

Native scaffolds, including collagen type I, hyaluronan, chitosan and alginate present a more natural micro-environment for the mesenchymal stem cells than synthetic scaffolds do.[25]

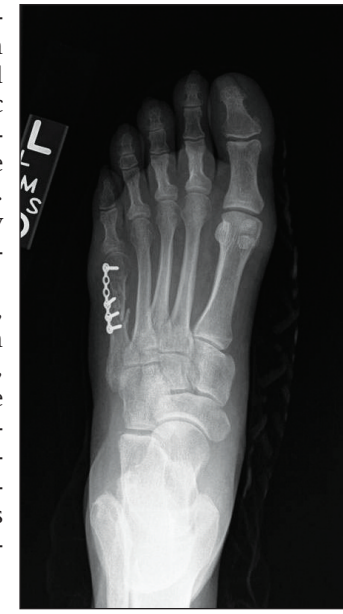

Figure 1: X-ray showing delayed union at 5 months.

Collagen Type I Hydrogels

Collagen type I hydrogels have several advantages. These matrices are biodegradable, can be metabolized by mesenchymal stem cells via the action of endogenous collagenases, elicit minimal, if any, inflammation, and surround the mesenchymal stem cells in three dimensions. The material properties of collagen hydrogels are similar to those of hyaline cartilage. Collagen gels can also be adapted as desired to most defect shapes.[26]

Trinity Evolution has an addition of a cancellous bone matrix that functions as a scaffold.

Compared with meshes or fleeces, in which cell seeding is often limited to superficial regions of the scaffold material, hydrogels permit a more even distribution of seeded mesenchymal stem cells, which promotes homogeneous production of extracellular matrix.[27]

The first results for use of transplanted mesenchymal stem cells seeded within collagen type I hydrogels to repair isolated, full-thickness, cartilage defects in humans was reported by Wakitani, et al. Two patients with a patellar defect were treated with collagen gels containing mesenchymal stem cells, which were covered with a periosteal flap. Fibrocartilaginous filling of the defects was found after one year, and both patients showed significantly improved clinical outcomes in their respective follow-ups after one, four, and five years.[28]

Rheumatoid Arthritis

Another area of research is treating rheumatoid arthritis, both the physical and immune aspect of the disease. Although it is still debatable, rheumatoid arthritis is believed to be a T cell-driven inflammatory synovitis disease in which T cells and synoviocytes participate in a complex network of cell- and mediator-driven events leading to joint destruction.

Joint destruction in rheumatoid arthritis and the anti-inflammatory and immune-suppressive properties of mesenchymal stem cells suggest that rheumatoid arthritis may be a

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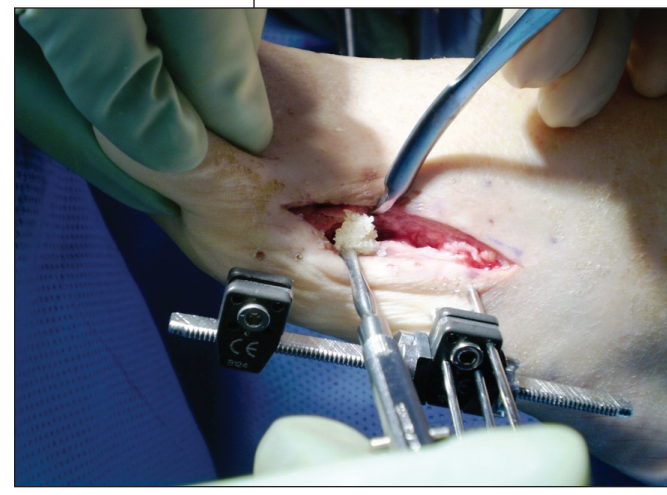

Figure 2: Application of stem cells in non-union site.

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candidate disease for cartilage and bone repair using mesenchymal stem cells therapy.

Mesenchymal stem cells can also inhibit the proliferation of autologous and allogeneic peripheral blood mononuclear cells in a dose-dependent manner. The inhibition was observed with mesenchymal stem cells and peripheral blood mononuclear cells, either from healthy donors or from patients suffering from autoimmune diseases.[29]

Bone Healing

The gold standard of bone grafts is the cancellous autograft, which is traditionally harvested from the patient's iliac crest. The iliac crest is the ideal donor site because it contains mesenchymal stem cells which are osteogenic, osteoinductive and osteoconductive. Even though an autograft is the gold standard, there are some challenges that can occur with using an iliac crest graft. These issues include a limited supply of donor material, donor site morbidity/pain, and the variable amounts or inconsistent amounts of mesenchymal stem cells.[30]

Most allografts do not have all three properties of autografts. For instance, demineralized bone matrix is osteo-inductive and stimulates the fusion of bone due to the bone morphogenetic proteins that induce or stimulate the mesenchymal stem cells. Synthetics materials such as ceramic hydroxyapatite are osteoconductive and facilitate bony ingrowth only.

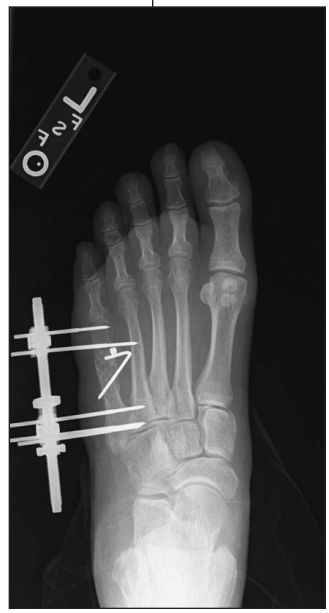

Figure 3: Post-operative x-ray.

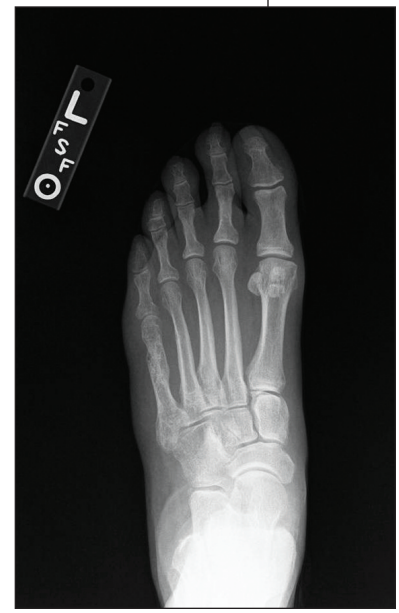

Figure 4: 12 week post-operative x-ray showing complete union.

Trinity Evolution

Currently, there are only two commercially-available mesenchymal stem cell products available on the market. Osteocel by Nuvasive is marketed to spinal surgeons. Trinity Evolution is marketed by Orthofix and the MTF foundation. These cells have undergone a selective depletion of immunogenic cells. The product also has an addition of a cancellous bone matrix that functions as a scaffold. The graft has all three properties of an autograft. It is osteoinductive, osteoconductive, and osteogenic.

> ***Adult mesenchymal stem cells offer promising results for many medical and surgical conditions.***

The production of Trinity Evolution begins within 96 hours post-mortem and is completed within 24 hours. Donors are screened and tested for over 50 diseases and illnesses. The tissue is frozen within one hour after processing. The health of the cell is best controlled by freezing slow and thawing fast. Rapid freezing can obviously damage the cells. The cells are stored at -80 degrees. Every lot is tested for viability and osteogenicity. Each lot of Trinity Evolution has a minimum of 250,000 cells per cc. There is greater than 70% cell viability along with in-vitro osteogenic capacity.

Before implantation, Trinity Evolution is thawed in a warm saline bath between 35-39 Celsius for roughly 30 minutes. The jar should remain in solution until contents flow freely upon inversion. The cryoprotectant is decanted, and D5 lactated ringers is added until used. The D5LR is decanted off before being implanted.

Rush, et al. published a retrospective analysis of revisional foot and ankle surgery in the Journal of Foot and Ankle Surgery.[31] In this study, 23 patients were reviewed who had implantation of mesenchymal stem cells after revisional foot or ankle surgery. A radiographic review was performed and there was radiographic new bone formation at the areas of implantation and a 91.3% union rate was observed. Statistical tests were performed to determine whether gender, diabetes, chronic renal insufficiency, or smoking were associated with time to healing. These statistical tests did not show any influence in time to heal.

Case Presentation

A sixty year old female presented to the clinic with a chief complaint that she fell off a four foot step ladder. She stated that she went to the local emergency room where x-rays were taken. The x-rays revealed a comminuted mid-shaft 5th metatarsal fracture of the left foot. Due to the severity of the fracture, open reduction internal fixation was determined to be the procedure of choice.

A complete history and physical was performed. Significant findings were thrombocytopenia, Raynaud's disease, coronary artery disease, and a 50 year pack-a-day year histo-

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ry of smoking. Medications included aspirin, atenolol, bupropion, etodolac, felodipine, furosemide, lorazepam, omeprazole, and pravastatin. Her lower extremity exam was unremarkable, except for ecchymosis and edema present at the fracture site on the left foot.

Surgery was performed which included ORIF, utilizing plate fixation. The patient was put in a non-weight bearing cast for six weeks. Serial x-rays were taken on a bi-weekly basis. A pulsed electromagnetic field bone stimulator was dispensed at eight weeks post-op due to slow consolidation of the fracture site. At five months post-op, it was determined that there was a delayed union which would most likely go on to a non-union (Figure 1).

The delayed non-union was most likely due to the patient's continued use of nicotine. The patient was brought back to surgery where the plate was removed, and Trinity mesenchymal (Figure 2) stem cells were put in the non-union site after the fibrous tissue within the site was removed. An external fixation device was applied and the patient was put in a non-weight bearing cast (Figure 3). Serial x-rays were taken and complete union was seen at approximately 12 weeks (Figure 4).

Conclusion

Adult mesenchymal stem cells offer promising results for many medical and surgical conditions. Even though recent research has shown promising results for foot and ankle surgery, further research still needs to be done. ■

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EXAMINATION

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See answer sheet on page 219.

1) Which one of the following markers is not found on an adult mesenchymal stem cell?
- A) Cd29
- B) Cd44
- C) Cd45
- D) Cd 120

2) Class II surface antigens are required to launch a reaction for:
- A) Antihistamines
- B) IGe
- C) T-cells
- D) Macrophages

3) Adult mesenchymal stem cells are:
- A) Multipotent
- B) Unipotent
- C) Totipotent
- D) Tripotent

4) An adult mesenchymal stem cell can NOT differentiate into a/an:
- A) Adipose
- B) Cartilage
- C) Neutrophil
- D) Bone

5) Adult mesenchymal stem cells are derived from the:
- A) Endoderm
- B) Mesoderm
- C) Ectoderm
- D) Tetraderm

6) When an adult mesenchymal stem cell expands via mitosis it forms a/an:
- A) Blastema
- B) Neutrophil
- C) Osteoclast
- D) Fibrin

7) 1 cc. of bone marrow aspirate from the iliac crest yields approximately:
- A) 10 mesenchymal stem cells
- B) 100 mesenchymal stem cells
- C) 1,000 mesenchymal stem cells
- D) 10,000 mesenchymal stem cells

8) As we age, the amount of mesenchymal stem cells:
- A) Stay the same
- B) Decrease
- C) Increase
- D) Turn into hemopoetic stem cells

9) When mesenchymal stem cells turn into osteoblasts, they are stimulated by:
- A) Osteoclasts
- B) Transforming growth factor beta
- C) Calcium
- D) Bone morphogenetic proteins

10) Mesenchymal stem cells are stored throughout the body as:
- A) Osteocytes
- B) Chondrocytes
- C) Pericytes
- D) Leukocytes

11) In which one of the following conditions are mesenchymal stem cells not used?
- A) Chronic lymphocytic leukemia
- B) Crohn's disease
- C) Rheumatoid arthritis
- D) graft vs. host

12) In Crohn's disease, what percentage reduction of symptoms did patients get after receiving mesenchymal stem cells?
- A) 20%
- B) 40%
- C) 80%
- D) 100%

13) When patients with myocardial infarction were treated with mesenchymal stem cells, there was a decrease in arrhythmias and also:
- A) increase in new blood vessel formation
- B) increase in infarctions
- C) decrease in alkaline phosphatase
- D) pulmonary edema

14) There has been published evidence that difficulty can arise when implanting mesenchymal stem cells in the heart because they can differentiate into:
- A) Cardiomyocytes
- B) Leukocytes
- C) Osteoblasts
- D) Keratinocytes

15) The mesenchymal stem cell that has the highest potential for chondrogenesis is derived from:
- A) synovium
- B) bone
- C) muscle
- D) adipose

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EXAMINATION

(cont'd)

16) Which one of the following is not considered a native scaffold for delivering stem cells?

A) Collagen
B) Alginate
C) Alpha hydroxyl polyester
D) Hyaluaron

17) The ideal scaffold for delivering adult mesenchymal stem cells for regeneration of cartilage should be:

A) Porous
B) Biodegradeable
C) Biocompatible
D) All the above

18) The ideal donor bone should have the following characteristics:

A) osteoinductive
B) osteoconductive
C) osteogenic
D) all of the above

19) Commercially-available mesenchymal stem cells that are used for bone have _____ added to them?

A) cortical bone
B) cancellous bone
C) bone morphogenetic protein
D) hydroxyappetite

20) Commercially-available bone mesenchymal stem cells have:

A) 25 stem cells per cc
B) 250 stem cells per cc
C) 25,000 stem cells per cc
D) 250,000 stem cells per cc

See answer sheet on page 219.

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Enrollment/Testing Information and Answer Sheet

Continuing Medical Education

Note: If you are mailing your answer sheet, you must complete all info. on the front and back of this page and mail with your credit card information to: ***Podiatry Management*, P.O. Box 490, East Islip, NY 11730.**

TESTING, GRADING AND PAYMENT INSTRUCTIONS

(1) Each participant achieving a passing grade of 70% or higher on any examination will receive an official computer form stating the number of CE credits earned. This form should be safeguarded and may be used as documentation of credits earned.

(2) Participants receiving a failing grade on any exam will be notified and permitted to take one re-examination at no extra cost.

(3) All answers should be recorded on the answer form below. For each question, decide which choice is the best answer, and circle the letter representing your choice.

(4) Complete all other information on the front and back of this page.

(5) Choose one out of the 3 options for testgrading: mail-in, fax, or phone. To select the type of service that best suits your needs, please read the following section, "Test Grading Options".

TEST GRADING OPTIONS

Mail-In Grading

To receive your CME certificate, complete all information and mail with your credit card information to:

Podiatry Management
P.O. Box 490, East Islip, NY 11730

There is **no charge** for the mail-in service if you have already enrolled in the annual exam CPME program, and we receive this exam during your current enrollment period. If you are not enrolled, please send $20.00 per exam, or $139 to cover all 10 exams (thus saving $61* over the cost of 10 individual exam fees).

Facsimile Grading

To receive your CPME certificate, complete all information and fax 24 hours a day to 1-631-563-1907. Your CPME certificate will be dated and mailed within 48 hours. This service is available for $2.50 per exam if you are currently enrolled in the annual 10-exam CPME program (and this exam falls within your enrollment period), and can be charged to your Visa, MasterCard, or American Express.

If you are *not* enrolled in the annual 10-exam CPME program, the fee is $20 per exam.

Phone-In Grading

You may also complete your exam by using the toll-free service. Call 1-800-232-4422 from 10 a.m. to 5 p.m. EST, Monday through Friday. Your CPME certificate will be dated the same day you call and mailed within 48 hours. There is a $2.50 charge for this service if you are currently enrolled in the annual 10-exam CPME program (and this exam falls within your enrollment period), and this fee can be charged to your Visa, Mastercard, American Express, or Discover. If you are not currently enrolled, the fee is $20 per exam. When you call, please have ready:

1. Program number (Month and Year)
2. The answers to the test
3. Your social security number
4. Credit card information

In the event you require additional CPME information, please contact PMS, Inc., at **1-631-563-1604**.

ENROLLMENT FORM & ANSWER SHEET

Please print clearly...Certificate will be issued from information below.

Name _____ Soc. Sec. #_____
Please Print: FIRST MI LAST

Address_____

City_____State_____Zip_____

Charge to: _____Visa _____ MasterCard _____ American Express

Card #_____Exp. Date_____

Note: Credit card is the only method of payment. Checks are no longer accepted.

Signature_____Soc. Sec.#_____Daytime Phone_____

State License(s)_____Is this a new address? Yes_____ No_____

Check one: _____ I am currently enrolled. (If faxing or phoning in your answer form please note that $2.50 will be charged to your credit card.)

_____ I am not enrolled. Enclosed is my credit card information. Please charge my credit card $20.00 for each exam submitted. (plus $2.50 for each exam if submitting by fax or phone).

_____ I am not enrolled and I wish to enroll for 10 courses at $139.00 (thus saving me $61 over the cost of 10 individual exam fees). I understand there will be an additional fee of $2.50 for any exam I wish to submit via fax or phone.

Over, please

ENROLLMENT FORM & ANSWER SHEET (cont'd)

EXAM #5/10
The Use of Adult Mesenchymal Stem Cells in Reconstructive Foot Surgery (Kimmel)

Circle:

1.	A	B	C	D	11.	A	B	C	D
2.	A	B	C	D	12.	A	B	C	D
3.	A	B	C	D	13.	A	B	C	D
4.	A	B	C	D	14.	A	B	C	D
5.	A	B	C	D	15.	A	B	C	D
6.	A	B	C	D	16.	A	B	C	D
7.	A	B	C	D	17.	A	B	C	D
8.	A	B	C	D	18.	A	B	C	D
9.	A	B	C	D	19.	A	B	C	D
10.	A	B	C	D	20.	A	B	C	D

LESSON EVALUATION

Please indicate the date you completed this exam

_____

How much time did it take you to complete the lesson?

_____ hours _____minutes

How well did this lesson achieve its educational objectives?

_____Very well _____Well

_____Somewhat _____Not at all

What overall grade would you assign this lesson?

A B C D

Degree_____

Additional comments and suggestions for future exams:

_____

_____

_____

_____

_____

_____