Stem Cells and Growth Factors in Wound Healing

Here's an update on advances in the field.

BY MATTHEW GAROUFALIS, DPM

Goals and Objectives

After completing this CME, the reader will:

Understand the physiologic dynamics of a chronic wound.

Understand the 4-week model of healing diabetic wounds.

Understand the importance and delivery of growth factors and stem cells in wound healing.

Understand the challenges of delivering growth factors to the wound.

Understand the role of amniotic membranes in healing chronic wounds.

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*Following this article, an answer sheet and full set of instructions are provided (pg. 138).—**Editor***

In chronic wounds, venous leg ulcers, and diabetic foot ulcers, there are often roadblocks to healing via traditional healing methodologies. Growth factor, stem cells, platelet rich plasma, and amniotic membranes now play an important role in how we can heal these wounds faster and with less morbidity than we have been able to do in the past.

In chronic wounds, we are faced with some very serious issues, especially in a diabetic patient. Persistent inflammation is common in all chronic wounds. We have excessive proteolysis cytokine receptor deficiencies, impaired progenitor cell recruitment, impaired angiogenesis, and of course delayed re-epithelialization of the wound. As we try to restore that healing, we first need to improve perfusion. Once we improve profusion and increase angiogenesis, we begin to normalize the wound environment. We can then use different grafts or healing materials to help push these cells towards healing.

Phases of Wound Healing

The three phases of normal wound healing are well known. They are the inflammatory phase, the proliferative phase, and the remodeling phase. The inflammatory phase typically is 0 to 46 days, depending on the injury. The proliferative phase is from day two to week three, depending on the age of the patient. The remodeling phase is from a period of about three weeks after the wounding or the injury, and that can take as long as a year.

In each of these phases, there are some changes in the chronic wound that we need to be aware of as we treat these patients. In the inflammatory phase, there's a decrease in the functional capacity of neutrophils and macrophages and there's an increase in the production of matrix metalloproteinases (MMPs), which can be very tissue-destructive. Growth factors

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are released slowly or halted. This results in an overall slowing of the entire inflammatory process; many times in a chronic wound, that is the only phase that we are in.

If we still have a chronic wound that gradually moves to the proliferative phase, we continue to face issues with angiogenesis, delayed response to growth factors, decreased fiberglass function, increased MMPs, and a decreased granulation tissue formation phase that could impair the final epithelialization. When we get to the remodeling phase and this patient is still in a state of chronicity, we continue to deal with decreased angiogenesis and decreased collagen production, which could result in scarring, poor matrix remodeling, and decreased wound tensile strength. This is important because it could result in re-ulceration. The risk factors for non-healing wounds include infection, pro-inflammatory cytokines, high levels of MMPs and proteases, low mitogenic activity, and senescent cells. We know that chronic wounds have failed to proceed through the orderly and timely repair process.

It's estimated that more than six and a half million patients in the U.S. alone are affected with chronic wounds. The impact is primarily seen in patients with underlying conditions that include diabetes, and arterial and cardiovascular disease. We know that 1-2% of the population in developed countries will experience a chronic wound, and a much higher percentage in underdeveloped countries. More than 25 billion dollars is spent annually in the treatment of chronic wounds. There are various treatment options that are available to treat these wounds, but many lack the key components necessary for the coordination of all phases of wound healing.

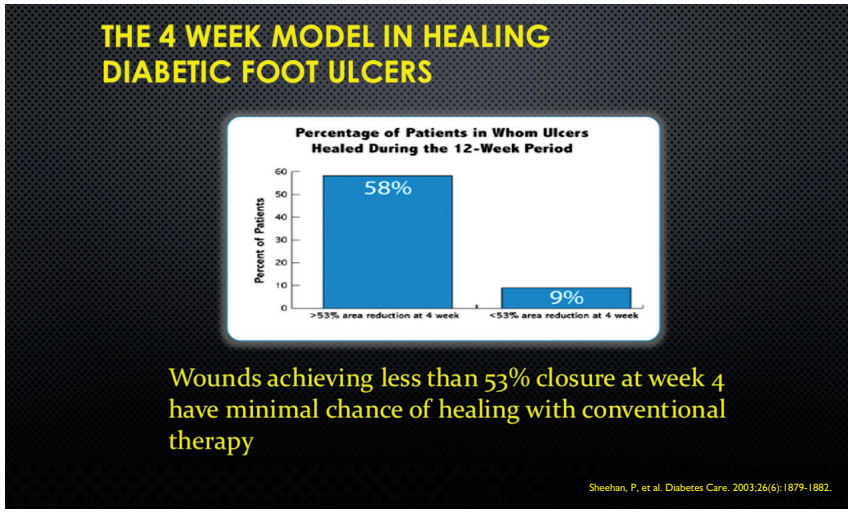

Figure 1: 4-week model

Venous Leg Ulcers

A venous leg ulcer is defined as a congestion of the venous system that leads to vascular permeability, causing local tissue damage from fibrin deposition, limiting oxygen and nutrients, trapping of growth factors, and the release of proteolytic enzymes. Venous leg ulcers account for 70-90% of all ulcers found in the lower leg. One percent or approximately two to three million adults in the U.S. have venous leg ulcers. Fifteen percent of these will never heal and the recurrence rate of those that do heal is close to 70%. The annual costs are up to five billion dollars for the care of these type of ulcers—that comes to about $40,000 per episode and up to two million workdays lost annually.

There are some interesting co-morbidities that are associated with venous leg ulcers and these include anemia, asthma, cellulitis of the lower leg, depression, diabetes, hypertension, osteoarthritis, pneumonia, UTIs, rheumatoid arthritis, congestive heart failure, and a complication of surgery including hip replacement. These are some things that we should be aware of as we treat these patients, and there are many algorithms for treatment. First and foremost, these typically include a venous reflux study, which should be done on every patient that has a venous leg ulcer. ABIs are mandatory and we often should include a biopsy as part of our standard initial treatment regime if these wounds have been long-standing.

Following that, these patients need compression therapy—the gold standard for venous leg wounds. A good standard wound care work-up and follow-through should be done to see if the patient has the potential to heal that wound. If good, standard wound care does not close the wound by at least 40% in four weeks, there is a need to go forward with advanced modalities and possibly even the use of a compression pump or pneumatic compression to help get these wounds closed.

Diabetic Foot Ulcers

Diabetic foot ulcers present many treatment obstacles. Dr. Jeff Robbins has said that diabetes could be viewed as a malignant disease. We look at the consequences of unhealed neuropathic ulcers and their five-year mortality rates, we can see that neuropathic ulcers, amputations, ischemic ulcers, and complications of peripheral arterial disease exceed some of the mortality rates of very common cancers such as prostate cancer, breast cancer, and some types of colon cancer. These mortality rates are 50% or higher than those with cancer and most of these patients with unhealed neuropathic ulcers could well be dead within five years. We know that unhealed neuropathic ulcers are one of the most common complications of diabetes. The annual incidence is up to 4% with the lifetime risk of up to 25%. Fifteen percent of all diabetic foot ulcers result in a lower extremity amputation, and 85% of lower limb amputations in patients with diabetes are preceded by ulceration. Mortality rates following amputation are up to 40% at one year and 80% at five years—norms for patients with this kind of a complication.

Peripheral neuropathy, of course, is a major contributing factor in diabetic foot ulcers. We know that the relationship between time to heal and the costs related to treatment of that ulcer

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go hand-in-hand. The longer it takes to heal that ulcer, the more costly it is going to be to get that ulcer healed.

Diabetic Metabolic Memory

There is a theory that may help us understand a little more clearly what is going on with the patient with diabetes. That theory, put forth by Dr. Ansgar Olson several years ago, is called Diabetic Metabolic Memory, and it states that irreversible changes occur at a cellular level when diabetes is uncontrolled. This results in continued faulty multicellular reproduction and outcomes. Continued less than appropriate cell reproduction leads to an escalating disabling cascade of factors; so metabolic memory is a phenomenon whereby diabetes complications persist and progress even after glycemic recovery is achieved.

This results in hyperglycemia-induced DNA hypermethylation and aberrant gene expression. These mutations that occur with mitosis during hyperglycemia are then replicated over and over again. So, think of your patients with a hemoglobin A1c of 13 or 14. They're in a chronic state of hyperglycemia and their cells, going through cell division every second, are dividing with mutations that are irreversible. This can easily account for some of the complications of diabetes that we see in terms of retinopathy, kidney disease, and of course, diabetic ulcers that are very difficult to heal. This can also account for the cognitive impairment that now goes hand-in-hand with diabetic foot ulcers. Notovitch in 2016 stated, "Individuals with diabetic foot ulcers were found to possess fewer cognitive resources than individuals without this complication, and this is present on their first diabetic foot ulcer incident." Remarkable. Keep this in mind with some of the patients that you are treating.

Venous leg ulcers account for 70% of all lower extremity ulcerations.

The Four-Week Model

When we treat diabetic foot ulcers, it's important to have a plan and a goal. The four-week model in the study by Peter Sheehan states that wounds achieving less than 53% closure by week four have minimal chance of healing with conventional therapy. Those ulcers that do not heal by 50% at week 4 have less than a 10% chance of healing by week 12. This theory (Figure 1) has been backed up by multiple studies by Bolton, Snyder, and Bloom. In other words, once we have debrided the wound, cleansed the wound, and made sure there was adequate perfusion to the wound, then we start counting four weeks and using good wound care.

Good wound care is not wet-to-dry dressings. Wet-to-dry dressings are well below the standard of care today. Good wound care is using collagens, calcium alginates, foams, and similar products that help close the wound. Good standard wound care, as well as offloading and possibly negative pressure, can be used also. If at the end of four weeks the wound is not closed by 50%, it is then time to go to the higher level of wound care—e.g., skin substitutes and some of the amniotic membranes or PRPs that will be discussed shortly. So the definition of good wound care is to treat the whole patient, not just the hole in the patient. We need to ensure adequate perfusion to the wound site, address metabolic challenges, make sure nutrition is where it should be, always debride to bleeding tissue, making sure of course that they are perfused, assess the patient's need for off-loading, compression and/or negative pressure, address the bioburden biofilms and infection. We need to rebalance the microscopic wound environment.

The wound healing mediators, the cytokines and chemokines, need to begin to work in order for us to reach 50% by four weeks. If they're not working, then we need to go to the next level. We have learned over the past few years that the different stages of wound healing require certain growth factors in order to make the process move. We know there is a set of growth factors in the inflammatory phase. There's a different set of growth factors that are more active in the proliferative phase and there's another different set of growth factors in the remodeling phase. They all need to be present and communicating in order for a wound to go through an orderly process and go from a chronic wound to a healed wound (Figure 2).

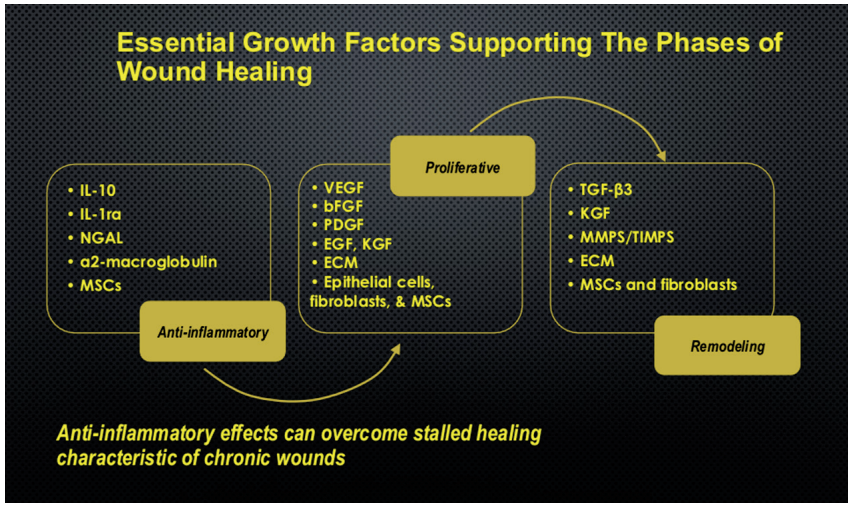

Figure 2: Essential growth factors

Stem Cells

Stem cells can be a great aid to healing chronic wounds. Normal wound healing requires the coordinated communication among cells, growth factors, and extracellular matrix proteins within the extracellular matrix. Stem cells and stem cell activators are

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the keystones to this process. They allow for a coordinated repair response. They also recruit other cells and they signal the release of growth factors and the appropriate MMPs into the extracellular matrix. What we are trying to achieve in a chronic wound is the re-establishment of dynamic reciprocity. Greg Schultz talked about this theory in 2011 where the extracellular matrix communicates with the cell signalers, and the signalers communicate with the cells that are going to be metabolically active in wound healing, and they all communicate with each other, creating the phenomenon of dynamic reciprocity (Figure 3).

In a chronic wound, this process does not exist. We do not have the ECM which contains laminin and elastin communicating with these chemokines, cytokines, and growth factors; and they do not communicate with the patient's active cells to create that circle of wound healing that we need to achieve. We now know that these mesenchymal stem cells that are present and recruited in the human body are at a very high level in a newborn. They then begin to drop off precipitously as we get older, becoming only a small fraction of what we had when we were newborns. We understand that newborns and infants heal differently than adults. They have a much more robust regenerative phase with a much lower active fibrosis phase than adults do. This is why infants and babies, when injured, heal without scarring and very quickly, whereas adults heal via scarring and less regeneration. The mesenchymal stem cells present in a newborn help create this response (Figure 4).

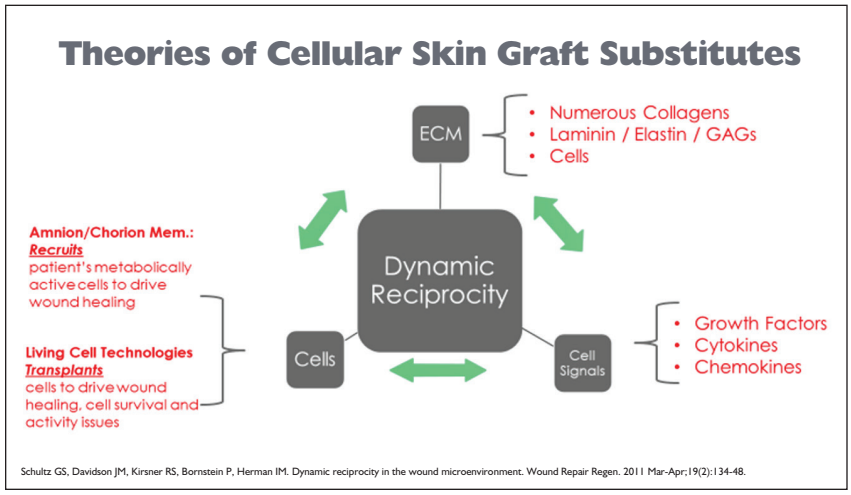

Figure 3: Dynamic reciprocity

It is essential for our own stem cells to get to the chronic wound; however, our own stem cells decrease rapidly with age. There's the autologous method using bone marrow, skin, hair follicles, and adipose as a method of getting stem cells to the wound. Your body can recruit them every time it's injured. However, that doesn't work so well in a chronic wound because recruitment doesn't happen effectively. In a chronic wound or in a compromised patient, there's little effective cellular communication. As a result, we depend upon PRP and, to a greater extent, amniotic membranes that cause mesenchymal progenitor cells to be recruited to the site of implantation.

PRP

Platelet-rich plasma (PRP) has been around for a while. It's used very often to help recruit stem cells. It is defined as a volume of plasma that has a platelet count above baseline, so it's typically spun down after the plasma is drawn. The amount of bioavailable growth factors depends on both the platelet storage and the release into the micro-environment. When these platelets regranulate after injection, growth factors are released and recruited, causing an inflammatory response that lasts about three days. As a result, fibroblasts accumulate and push the area into a proliferative phase so collagen can then begin to accumulate. There are several different PRP systems available for use. They use different protocols, so each has a different make-up of PRP formulations. Also, as we know now, PRP is extremely donor-dependent. You're going to have a much more robust PRP sample in a younger individual than you will in an older individual, especially if that older individual is immunocompromised. The reproducibility of PRP is not always possible so as a result we cannot always assume what the outcome will be by simply applying a PRP injection.

Amniotic Membranes

Amniotic membranes were first used as early as 1910 as a biologic scaffold for skin defects and burns. In the 1990s, amniotic membranes began to be used extensively for ophthalmic surgery, and they offered remarkable results with less scarring around the cornea in the eye. Today, vigorous screening of donors, cleansing, and sterilization must occur before use. The membrane is then processed and preserved in a variety of different formats, including cryopreservation, lyophilization, freeze-drying, and dehydration. Many of these formats include amnion-only products as well as amnion and chorion products. Today, an amnion-chorion product has the highest concentration of pluripotent cells, proteins, and growth factors available for implantation.

Why are amniotic membranes so important? They contain barrier properties because they come from the placenta. They do modulate inflammation, which is very important. In a wound that is stuck in the inflammatory process can be modulated, then we are able to change the structure of the wound. It does reduce scar tissue formation because of the growth factors present in vast quantities. By reducing the scar tissue formation, we get normal collagen formation, resulting in stronger, better-healed wounds with less of a chance for re-ulceration. Amniotic membranes are immunologically privileged, which allows for no rejection to occur. They contain essential growth factors and stem cells precursors. This is what enhances wound healing.

There are many different types of placental products available for use

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today. We'll examine a bi-layered product that consists of amnion and chorion. We know from a study from Tobb and Lin in 2015 that the amnion contains about 20% of the growth factors and the chorion contains about 80% of the growth factors, with the exception of epidermal growth factor, which is prominent in the amnion layer. So, when using just an amnion product, only 20% of the growth factors are available. If we have a chorion-only product, we have 80% of the growth factors. If we combine the layers, both amnion and chorion, we are getting all the available growth factors that we need to help heal that wound.

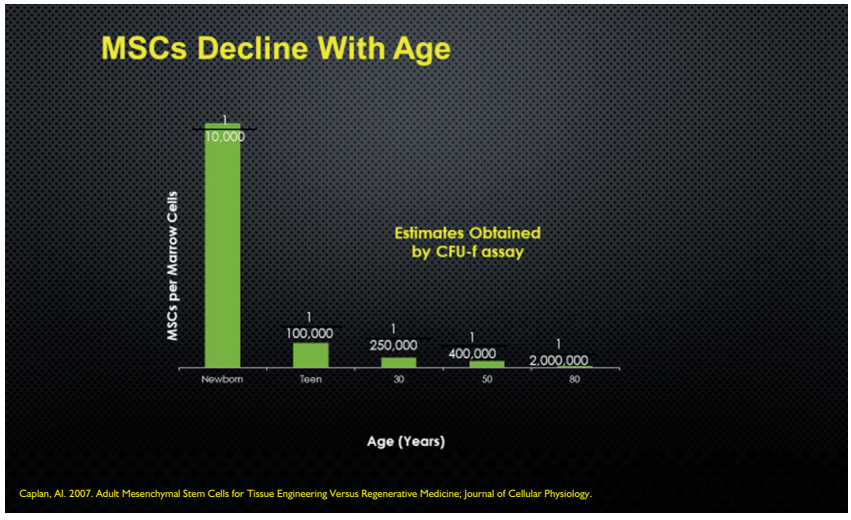

Figure 4: MSCs decline with age

A combination of amnion and chorion together deliver no fewer than 285 regulatory proteins to the wound. These include growth factors, chemokines, and cytokines, as well as angiogenic proteins. The extracellular matrix (ECM) components include collagen type 1, collagen type 4, hyaluronic acid, heparin sulfate, proteoglycan, fibronectin, and laminate, as well as numerous growth factors such chemokines, cytokines, proteases, and protease Inhibitors that are known to participate in the wound healing process.

Now we must deliver the stem cells into the wound. An autologous transfer can be done, but this usually requires at least some sort of a surgical procedure. However, advanced products such as the PRP, the amniotic membranes, and the umbilical cord products can act as recruiters, which help pull the body's own stem cells into the wound. There have been studies to show the migration of mesenchymal stem cells to the wound area.

There is an in-vivo MSC migration study on mice showing that with three areas of treatment, one with chorion-amnion membrane inserted subcutaneously, the area that results in the largest and fastest recruitment of stem cells is not the "sham" surgical incision with no implant; it is rather the area where the amniotic membrane has been placed.

One of the biggest issues with venous leg ulcers is the high percentage that never heal.

The Green Mouse Model

The model that has been used as described is the Green Mouse Model. One of a pair of mice used to generate the model system was genetically altered such that every cell in his body expressed a fluorescent protein called green fluorescent protein. This "green" mouse was surgically connected by a flap of dorsal skin to a dorsal skin flap of another mouse, which was genetically identical other than not expressing green fluorescent protein, so that its cells would now glow green.

Two weeks after the surgery to connect the two mice, the animals share their blood circulation. This is known as parabiosis, such that blood and other cells, including circulatory stem cells from each animal, flow into the other through blood circulation. We can then see these green cells begin to migrate across at a rate of speed that allows us to understand that amniotic membrane compared to any other product results in faster recruitment of mesenchymal stem cells to the wounded area. As a result, we now know the amnion-chorion membrane implant recruits stem cells and acts as a stem cell recruiter faster than if it were not used at all, and this helps these wounds heal much more quickly. We see that as soon as the amniotic membranes are implanted, they begin to signal.

Amniotic membranes signal fibroblasts, epithelial cells, hematopoietic stem cells, the bone marrow mesenchymal stem cells, and the adipose tissue-derived stem cells in both healthy and diabetic patients.

These membranes begin to both proliferate and migrate, biosynthesis occurs, and the wound begins to communicate and heal. At the same time, we now know the angiogenic properties and those growth factors that are important for angiogenesis. We know that vascular endothelial growth factor (VEGF) is present, but there is also leptin, epidermal growth factor (EGF), beta fibroblast growth factor (FGF), and many other growth factors and proteins that are important in angiogenesis that are included. So not only can healing of a wound be stimulated, but angiogenesis can be promoted also (Figure 5).

Antimicrobial/Antiviral Effects

There is another area of amniotic membrane that deals with antimicrobial and antiviral effects. The antibacterial effect is against some gram-positive cocci, streptococci, and streptococcus Aureus, and some gram-negative bacilli, E. coli, and pseudomonas. Amniotic membranes can also produce antimicrobial molecules such as bactricidine, beta-lysin, transferrin, and 7S globulin.

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These are important in helping to defeat what otherwise might be bacteria that can damage the healing process of a wound (Figure 6).

There is a robust future for stem cells and growth factors in wound care. There are few products available that contain anti-inflammatory growth factors, anti-scarring, and regenerative growth factors. We know also that there's a structural component that is very important in the extracellular matrix—a protective barrier is involved in the ECM, and it acts as a reservoir for growth factors and other cells. Sustained release of these growth factors is important. As a result, we deliver a response to an otherwise hostile wound environment by implanting these new structures into the area of the chronic wound.

The future of stem cells in wound care is in the early phases right now. We've had some experience with PRP for many years now. We are working with amniotic membranes and umbilical cord products and, of course, we have the autologous transfer of harvested stem cells. It is still a tricky surgical procedure but that may become easier in the future.

Conclusion

In *2001, A Space Odyssey,* Arthur C. Clarke writes, "The only way to discover the limits of the possible is to go beyond them to the impossible." What we have done in the past 10 years using many of these products has been remarkable. Ten years ago, we never would have thought that we could do these things, but we are now creating, using, and building upon these innovations. We continue to go forward and conquer the impossible. **PM**

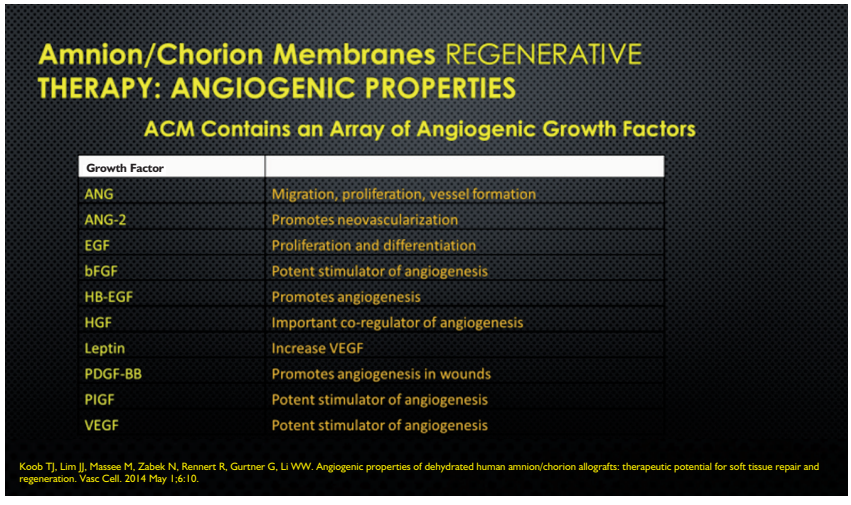

Figure 5: Angiogenic properties

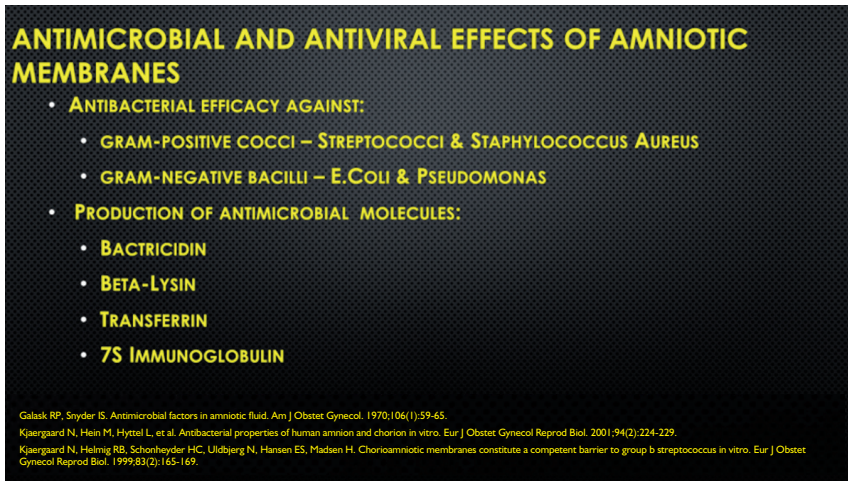

Figure 6: Antimicrobial properties

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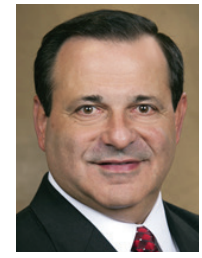

Dr. Garoufalis is board certified by the American Board of Podiatric Medicine, with the added qualification of Amputation Prevention and Limb Salvage, and by the American Board of Foot and Ankle Surgery. He is also a Certified Wound Specialist by the American Board of Wound Management. He is the past president of the American Podiatric Medical Association and the International Federation of Podiatrists. He has authored many articles and has lectured extensively across the country on a variety of topics pertinent to podiatric medicine, surgery, and practice management.

CME EXAMINATION

SEE ANSWER SHEET ON PAGE 139.

1) The 4-week model for healing diabetic foot ulcers calls for the wound to be closed by _____ at four weeks so as to have a reasonable chance for complete closure at 12 weeks.

- **A) 20%**
- **B) 40%**
- **C) 50%**
- **D) 70%**

2) As we age, our own stem cells _____ in number.

- **A) Increase**
- **B) Decrease**
- **C) Decrease until puberty, then increase**
- **D) Fluctuate every year**

3) In amniotic membranes, the chorion layer contains _____ of the growth factors relative to the amnion layer.

- **A) 40%**
- **B) 60%**
- **C) 10%**
- **D) 80%**

4) Venous leg ulcers account for _____ of all lower extremity ulcerations.

- **A) Less than 10%**
- **B) About 50%**
- **C) Greater than 70%**
- **D) 25%**

5) Diabetic Foot Ulcers

- **A) Have a high recurrence rate**
- **B) Can often be associated with diminished vascular supply**
- **C) Can lead to amputation**
- **D) All the above**

6) When debriding a wound, it is important to _____

- **A) Leave a bit of hyperkeratotic tissue around the wound edge**
- **B) Just wipe the wound with gauze**
- **C) Debride to bleeding tissue**
- **D) Never allow the wound to bleed**

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Continuing Medical Education

CME EXAMINATION

7) A combination of amnion and chorion together delivers no fewer than _____of the regulatory proteins to the wound.

A) 285
B) 385
C) 485
D) 685

8) In dynamic reciprocity:

A) The extracellular matrix communicates with the cell signalers
B) The stem cells communicate with the wound biofilm
C) The chemokines communicate with the fat cells
D) The MMPs communicate with the erythrocytes

9) One of the biggest issues with venous leg ulcers is:

A) The extremely high amputation rate
B) Venous leg ulcers account for 70-90% of all leg ulcers found in the lower leg.
C) Non-compressive dressings are required
D) They have a very low recurrence rate

10) Risk factors for non-healing wounds include:

A) Infection
B) High levels of MMPs
C) Senescent cells
D) All the above

SEE ANSWER SHEET ON PAGE 139.

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2. The answers to the test
3. Credit card information

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Name _____ Email Address _____
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Charge to: _____Visa _____ MasterCard _____ American Express

Card # _____ Exp. Date _____ Zip for credit card _____

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State License(s) _____ Is this a new address? Yes _____ No _____

Check one:

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Over, please

ENROLLMENT FORM & ANSWER SHEET *(continued)*

EXAM #6/22
Stem Cells and Growth Factors in Wound Healing
(Garoufalis)

Circle:

1.	A	B	C	D	6.	A	B	C	D
2.	A	B	C	D	7.	A	B	C	D
3.	A	B	C	D	8.	A	B	C	D
4.	A	B	C	D	9.	A	B	C	D
5.	A	B	C	D	10.	A	B	C	D

Medical Education Lesson Evaluation

Strongly agree [5]	Agree [4]	Neutral [3]	Disagree [2]	Strongly disagree [1]

1) This CME lesson was helpful to my practice ____

2) The educational objectives were accomplished ____

3) I will apply the knowledge I learned from this lesson ____

4) I will makes changes in my practice behavior based on this lesson ____

5) This lesson presented quality information with adequate current references ____

6) What overall grade would you assign this lesson?

A B C D

7) This activity was balanced and free of commercial bias.

Yes _____ No _____

8) What overall grade would you assign to the overall management of this activity?

A B C D

How long did it take you to complete this lesson?

_____hour _____minutes

What topics would you like to see in future CME lessons ? Please list :

_____

_____

_____

_____

_____