Stem cells and growth factors in healing the diabetic foot ulcer

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Article points

1. Growth factors and stem cells, platelet rich plasma and amniotic membranes are all important in the healing of diabetic foot ulcers
2. Using the 4-Week model for wound healing will act as a guide to taking appropriate steps to wound closure.
3. Understanding the microscopic needs of the wound environment is crucial to closure.

Key words

- Amniotic membranes
- Diabetic foot ulcers
- Growth factors
- Stem cells

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In diabetic foot ulcers, there are roadblocks to the traditional healing methodology. The importance of growth factor and stem cells, platelet rich plasma and the revolution of amniotic membranes now play a key role in how these wounds can be healed faster and with less morbidity than has been possible in the past. This article examines the importance of growth factors and stem cells, platelet-rich plasma and amniotic membranes in the healing of diabetic foot ulcers.

In people with diabetes, we are faced with some very serious issues. Persistent inflammation is common in all chronic wounds. There are excessive proteolysis cytokine receptor deficiencies, impaired progenitor cell recruitment, impaired angiogenesis and, of course, delayed re-epithelialisation of the wound. As clinicians try to restore that healing, first of all, there is a need to improve perfusion. Once profusion is approved and angiogenesis increased, the wound environment begins to normalise. Different grafts or healing materials can then be used to help push these cells towards healing.

The three phases of normal wound healing are well known. They are the inflammatory phase, the proliferative phase and the remodelling phase. The inflammatory phase is typically 0 days to 46 days, depending on the injury. The proliferative phase is from day 2 to week 3, depending on the age of the patient. The remodelling phase is from a period of about 3 weeks after the wounding or the injury and can take as long as a year. There are some changes in the chronic wound in each of these phases that clinicians need to be aware of as they treat these patients. In the inflammatory phase, there is a decrease in the functional capacity of neutrophils and macrophages and there is an increase in the production of matrix metalloproteinases (MMPs), which can be highly tissue destructive. Growth factors are released slowly or halted. This results in a generalised slowing of the entire inflammatory process, and often in a chronic wound, it does not proceed out of this phase. Should the clinician be faced with a chronic wound that gradually moves to the proliferative phase, they are still faced with angiogenesis, delayed response to growth factors, decrease fibreglass function, increased MMPs and a decreased granulation tissue formation phase that could impair the final epithelialisation. When we get to the remodelling phase and this patient is still in a state of chronicity, clinicians are still faced with decreased angiogenesis and decreased collagen production, which could result in scarring, poor matrix remodelling, and decreased wound tensile strength. This is important because it could result in reulceration. The risk factors for non-healing wounds include infection, pro-inflammatory cytokines, high levels of MMPs and proteases, and low mitogenic activity and senescent cells. In chronic wounds, we know that these are wounds that failed to proceed through the orderly and timely repair process.

It is estimated that more than six and a half million patients in the US alone are affected by a chronic wound (Sen et al, 2009). The impact primarily seen in patients with underlying conditions include diabetes, arterial disease and cardiovascular disease. A total of 1%–2% of the population in developed countries will experience a chronic wound at some point in their lives (Guest et al, 2015). More than US$25bn is spent annually in the treatment of chronic wounds (Sen et al, 2009). 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.

Diabetic foot ulcers present many treatment obstacles. It has been said by Dr Jeff Robbins et al (2008) that diabetes could be viewed as a malignant disease and, perhaps, it should be. If the consequences of unhealed neuropathic ulcers and their 5-year mortality rates are examined, we can see that neuropathic ulcers, amputations, ischaemic 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 (Armstrong et al, 2007). These mortality rates are 50% or higher most of these patients that have unhealed neuropathic ulcers could well be dead within 5 years (Armstrong et al, 2007). We know that it is one of the most common complications of diabetes. The annual incidence is up to 4% with the lifetime risk of up to 25% (Sanders, 1994; Reiber and Ledoux, 2002; Boulton et al, 2004). 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 (Pecoraro et al, 1990; Apelqvist and Larsson, 2000). Mortality rates following amputation of up to 40% at 1 year and 80% at 5 years are the norm with patients with this kind of a complication (Kirsner et al, 2015). 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 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 and that they are directly proportional to each other. There is a theory that may help us understand a little bit better what is going on with the patient with diabetes. This theory is called 'Diabetic Metabolic Memory' (Olsen et al, 2010). The theory states that there are 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 escalating disabling cascade of factors so metabolic memory is a phenomenon whereby diabetes complications persists and progress after glycaemic recovery is achieved. This results in hyperglycaemia-induced DNA hypermethylation and aberrant gene expression. These mutations that occur with mitosis during hyperglycaemia are then replicated over and over and over again.

If one thinks of their patients with a haemoglobin A1c of 13 or 14 and they are in a chronic state of hyperglycemia, their cells are going through cell division every second. Their cells 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. Natovitch et al (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." This should be borne in mind with some of the patients that clinicians are treating.

When diabetic foot ulcers are treated, it is important to have a plan and a goal. It is the author's preference to use the 4-week model in healing diabetic foot ulcers. This model, in the study by Sheehan (2003), states that wounds achieving less than 53% closure at 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% of healing by week 12. This theory has been backed up by multiple studies (Bolton et al, 2004: Synder et al, 2010; Blume et al, 2012) to show that this is a useful way to treat diabetic foot ulcers and venous leg ulcers using the 4-week protocol. In other words, once we have debrided the wound, cleansed the wound, made sure there was adequate perfusion to the wound, then we start counting 4 weeks, while adopting appropriate wound care.

Appropriate wound care is no longer wet-to-dry dressings. Wet-to-dry dressings are well below the required standard of care and should only be used for a very short period of time, according to many wound care experts. Appropriate wound care is using collagens, calcium alginates, foams and similar products that help close the wound. Effective standard wound care, as well as offloading and possibly negative pressure, can be used also. If at the end of 4 weeks the wound is not closed by 50%, it is then time to go to the higher level of wound care, such as skin substitutes and some of the amniotic membranes or platelet-rich plasma (PRP) that will discussed in this article shortly. So, the definition of appropriate wound care is to treat the whole patient, not just the hole in the patient, ensure

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adequate perfusion to the wound site, address metabolic challenges, ensure nutritional advice is provided, debride bleeding tissue, ensuring they are perfused, assess the patient's need for offloading, compression and/or negative pressure, and address the bioburden biofilms and infection.

The microscopic wound environment must be rebalanced. The wound healing mediators — the cytokines and chemokines — need to begin to work, in order for wound closure to reach 50% by 4 weeks. If they are 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 to make the process move. There is a set of growth factors in the inflammatory phase, such as IL-10, IL-1ra and a2-macroglobulin, a different set of growth factors that are more active in the proliferative phase, such as VEGF, PDGF, and fibroblasts, and yet another set of growth factors in the remodelling phase, such as TGF-3, KGF and MMPS/TIMPS, and they all need to be present and communicating for this wound to undertake an orderly wound healing process, and go from a chronic wound to a healed wound.

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 central in this process as they allow for a coordinated repair response. They also recruit other cells and 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. Schultz et al (2011) spoke about this theory, where the extracellular matrix communicates with the cell signalers and they communicate with the cells that are going to be metabolically active in wound healing, creating the phenomenon of dynamic reciprocity. In a chronic wound, this process does not exist. These wounds do not have the ECM that 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 must be achieved.

It is now apparent that the mesenchymal stem cells that are present and recruited in the human body are at a very high level as a newborn. They then begin to drop off precipitously with age. 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, which is why infants and babies that are injured heal without scarring and very quickly, whereas adults heal via scarring and less regeneration. The mesenchymal stem cells present in a newborn help to affect this response.

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. The body can recruit them every time it is injured. However, that does not work so well in a chronic wound as recruitment does not happen effectively. In a wound that is chronic or in a compromised patient, there is little effective cellular communication. As a result, the body depends upon PRP and, to a greater extent, amniotic membranes that cause mesenchymal progenitor cells recruitment to the site of implantation. Perhaps with regards to some of the metabolic memory issues mentioned earlier, this is how we make up for that. PRP and, to a greater extent, the amniotic membranes, are the cause of mesenchymal progenitor cells that actually recruit to the site of implantation.

PRP is very often used to help recruit these stem cells. It is defined as a volume of plasma that has a platelet count above baseline, so it is 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 microenvironment. 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 where collagen can begin to accumulate. There are several different PRP systems available for use. They use different protocols, so each has a different makeup of PRP formulations. Also, PRP is extremely donor dependent. A more robust PRP sample will be found in a younger individual than an older one, especially if that older individual is immunocompromised. The reproducibility of PRP is not always possible so, as a result, the outcome cannot always be assumed.

Amniotic membranes were first used as early as 1910 (Koob et al, 2013) as a biologic scaffold for skin defects and burns. In the1990s, amniotic membranes were used extensively for ophthalmic surgery and

they offered remarkable results with less scarring in the cornea. At present, vigorous screening of donors cleansing and sterilisation must occur before the use of amniotic membranes. The membrane is then processed and preserved in a variety of different formats, including cryopreservation, lyophilisation, 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 (Koob et al, 2015a).

So why are amniotic membranes so important? They contain barrier properties as they come from the placenta. They modulate inflammation, which is important, and in a wound that is stuck in the inflammatory process, if this can be modulated, then the structure of the wound can be changed. They reduce scar tissue formation because of the growth factors present in vast quantities. By reducing the scar tissue formation, normal collagen formation is created, resulting in stronger, better healed wounds with less of a chance of reulceration. They are immunologically privileged, which allows for no rejection to occur. They also 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 today. A study by Koob et al (2015a) showed that amnion contains about 20% of all growth factors and the chorion contains about 80% of all growth factors, with the exception of epidermal growth factor, which is prominent in the amnion layer. Therefore, when using an amnion-only product, the patient is only getting 20% of the growth factors available. If a chorion-only product is used, the patient is only getting 80% of the growth factors. If the layers are combined, the patient gets all the available growth factors that are needed to help heal that wound. A combination of amnion and chorion together deliver no less than 285 regulatory proteins to the wound; these include chemokines and cytokines, as well as angiogenic proteins (Koob et al, 2015b). The 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. They also are contained inhibitors of MMPs that outnumber inhibitors by a ratio of 28 to 1 (Lei et al, 2017).

Stem cells must now be delivered into the wound. An autologous transfer can be conducted, but this usually requires at least some sort of a surgical procedure. However, there are advanced products, the PRP, the amniotic membranes, and the umbilical cord products now, that can act as recruitments. Recruiters help pull the body's own stem cells into the wound. These products help to modulate stem-cell recruitment. Stem cells in the amniotic fluid and membrane do not survive processing typically. Their remnants do activate the patient's own stem cells to be recruited. Should some stem cells withstand processing, they typically succumb to the hostile environment before they can differentiate. There have been studies to show the migration of mesenchymal stem cells to the area of wounding.

There is an in vivo MSC migration study on mice that shows that when this animal has 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' — the surgical incision with no implant — it is the area where the amniotic membrane has been placed. The model that has been used as described is the Green Mouse Model (Koob et al, 2013). One of a pair of mice used to generate the model system was genetically altered so that every cell in its body expressed a fluorescent protein called green fluorescent protein. This green mouse with surgically connected by 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 glow green. Two weeks after the surgery to connect the two mice, they shared their blood circulation, known as parabiosis, with blood and other cells, including circulatory stem cells from each animal, flowing into the other through blood circulation. These green cells begin to migrate across at a speed that allows us to understand that amniotic membrane results in faster recruitment of mesenchymal stem cells to the wounded area, when compared to any other product.

As a result, it is clear that the amnion-chorion membrane acts as a faster stem cell recruiter than if it was not used at all and this helps these wounds heal at a faster rate. As soon as they are implanted, they begin to signal. They signal fibroblasts, epithelial cells, hematopoietic stem cells, the bone marrow

Conflict of interest

There is no conflict of interest. Some brand names have been used from time to time for clarity purposes only. Due to the presence of different branded versions of the same agent potentially, there is no product promotion or recommendation that should be inferred by this.

mesenchymal stem cells, and the adipose tissue derived stem cells in both healthy people and in people with diabetes to begin to proliferate and migrate. Biosynthesis then occurs and the wound begins to communicate and heal. At the same time, the angiogenic properties and those growth factors that are important for angiogenesis, like VEGF, are present, but there is also leptin, EGF, beta FGF and many others growth factors and proteins that are important in angiogenesis that are included. Healing of a wound can be stimulated, while angiogenesis can be promoted also.

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

There is a robust future of 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 that in the extracellular matrix, there is a structural component that is very important; the ECM. A protective barrier is involved in extracellular matrix and it acts as a reservoir for growth factors and other cells. Sustained release of these growth factors is important and, as a result, we have delivered a response to 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 have had some experience with PRP for many years and we are just getting started with amniotic membranes and umbilical cord products. We also have the autologous transfer of harvested stem cells. It is still a tricky surgical procedure, but that may become easier in the future.

That brings us to our closing and there is a quote that speaks a little bit as to where we are in this odyssey. It is from Arthur C. Clarke's *Profiles of the Future: An Inquiry into the Limits of the Possible*: "The only way to discover the limits of the possible is to go beyond them to the impossible." What we have done over 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. It was thought they were not possible but, by using these products, many things are now possible, so we continue to go forward and attempt to conquer the impossible. ■

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Expert commentary: Stem cells and growth factors in healing the diabetic foot ulcer

The philosopher and author George Santayana (1917) wrote: "Those who cannot remember the past are condemned to repeat it." While this is widely used to discuss conflict and wars, I suspect we are in the same situation with growth factors, skin substitutes and amnion chorion complex and diabetes foot ulcer healing. I was actively using growth factors and dermal replacement therapy in the late 90s in my clinic, but these products stopped being available in the UK due to concerns about efficacy and, in particular, about price. In truth, the products were never commercially viable in the UK.

No one would deny that chronic wounds and diabetes-related foot ulceration are a huge drain on NHS resources. However, at a time when we are struggling to fund even standard care then such expensive products with limited applicability are unlikely to have a future in the UK. Indeed, beyond research and certain highly specialised centres in the UK, there are no products widely available to foot care teams in the UK (NICE, 2018). The cost of these products, despite widely optimistic financial modelling is rarely justifiable and the upfront cost, invest to save model is only going to work if the largely fixed costs, people, buildings etc can be reduced. The marginal costs, dressings etc are not large enough to be offset and the social costs are not offset by the NHS budget. Even amputations and rehabilitation costs are in a different budget from the clinic paying thousands to use these products.

I would love a technological, biological solution to come to our rescue in overworked clinics, which are sometimes unsuccessful in healing a foot ulcer. At present, not every NHS trust has a foot service and within those that do, basic skills, such as pressure redistribution, infection and vascular intervention, are still not being used fully effectively. Therefore, unless the cost and availability of these products becomes much more NHS-friendly, I do not see them having a wide application in the UK for many years to come. ■

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