ELSEVIER

The Next Generation of Burns Treatment: Intelligent Films and Matrix, Controlled Enzymatic Debridement, and Adult Stem Cells

H. Drago, G.H. Marín, F. Sturla, G. Roque, K. Mártire, V. Díaz Aquino, R. Lamonega, C. Gardiner, T. Ichim, N. Riordan, J.C. Raimondi, S. Bossi, A. Samadikuchaksaraei, M. van Leeuwen, J.M. Tau, L. Núñez, G. Larsen, R. Spretz, and E. Mansilla

ABSTRACT

We describe a novel technology based on nanoengineered multifunctional acellular biologic scaffolds combined with wound dressings and films of the same kind. This method allows selective delivery and release of shielded biomaterials and bioactive substances to a desired wound or damaged tissue while stimulating the selective anchoring and adhesion of endogenous circulating repairing cells, such as mesenchymal stem cells, to obtain a faster and more physiologic healing process. We also present a new controlled enzymatic debridement process for more effective burned tissue scarolysis. In light of our preliminary in vitro and in vivo data, we are convinced that these approaches can include the use of other kinds of adult stem cells, such as endometrial regenerative cells, to improve the vascularization of the constructs, with great potential in the entire tissue and organ regeneration field but especially for the treatment of severely burned patients, changing the way these lesions may be treated in the future.

ALTHOUGH mortality in patients with extensive deep burns is decreased by early surgical scarectomy, autografting, cadaver skin implantation, skin substitutes, and intensive care units, it remains unacceptably high in the light of the advancements of the 21st century. Prolonged hospitalization times, multiple and sometimes excessively large surgical procedures, too many infectious complications, severe functional and esthetic chronic defects, and expensive treatment costs are other common results of standard therapeutic techniques actually in use in most burns units around the world.[1] A new paradigm of treatment is urgently needed.

CURRENT TREATMENT LIMITATIONS AND PROPOSED APPROACH

Achieving permanent replacement of skin in full- and deep–partial-thickness burn injuries and other chronic wounds remains a complicated and not yet resolved problem.[2] Autografting has been the gold standard for burn treatments, but many times there is not sufficient healthy skin, and this procedure produces new lesions on the patient. Cultured epithelial autografts (CEA) have been used to resurface large areas of skin loss. Keratinocytes are undoubtedly a troublesome type of cell to obtain, culture, and expand.[3] Residual dermis or dermal elements are essential for minimization of contraction and scar formation. When the lesion is so deep that no dermal elements can be found, no simple epithelial graft can heal the defect. In these cases a neomatrix is needed. Some groups have implanted CEA over allogenic cadaver skin or acellular cadaver dermis or have tried to simultaneously deliver human keratinocytes cultured over a fibrin sealant or other biodegradable scaffolds as a carrier and matrix vehicle.[4,5] Tissue engineering composite grafts resembling human skin have also been attempted by various researchers. Also, various synthetic or biologic dermal analogs have been studied in animals and humans.[6] There is a lack of description involving acellular scaffolds of porcine origin with all

From the Banco de Tejidos (H.D., F.S., S.B.), Hospital de Quemados, CABA, Argentina; Laboratorio de Ingeniería Tisular (G.H.M., G.R., K.M., V.D.A., J.C.R., J.M.T., E.M.), Medicina Regenerativa y Terapias Celulares, and Servicio de Quemados y Banco de Piel (R.L., C.G.), CUCAIBA, Hospital San Martín, Ministerio de Salud de la Provincia de Buenos Aires, La Plata, Argentina; Medistem Inc. (T.I., N.R.), San Diego, California, USA; Imperial College (A.S.), London, Great Britain; Faculty of Medicine (M.v.L.), Vrije Universiteit, Amsterdam, The Netherlands; Nanovogue Corp. and University of Chicago (L.N.), Illinois, USA; and LNK Chemosolutions and University of Nebraska (G.L., R.S.), Lincoln, USA.

360 Park Avenue South, New York, NY 10010-1710

0041-1345/10/$–see front matter
doi:10.1016/j.transproceed.2009.11.031

three-dimensional configurations for skin regeneration with nanoparticles and/or fibers (NPFs) with at least one characteristic diameter of 200–300 nm and a length in the range of 3–0.03 μm, coated with specific monoclonal antibodies to promote homing of tissue-regenerating stem cells, such as mesenchymal stem cells (MSCs). Loading with bioactive agents and other drugs to stimulate wound healing is also possible. MSCs have great plasticity, and increasing evidence suggests their use in skin regeneration.[7] The tissue-engineered dermal equivalents lack blood vessels and may act as possible barriers against nutrients necessary for keratinocyte or stem cell survival on top of such composites. The additional period required to reperfuse the skin substitute increases the ischemia time and decreases the nutrition and immune functions of healing tissues as well as increases the possibility of microbial colonization and graft destruction.[8]

In this regard the combination of these substitutes not only with MSCs but also with endometrial regenerative cells (ERCs), bearing an intrinsically strong capacity to build new vessels, surely represents a good option to accelerate vascularization especially for skin wounds with deficient blood supply.[9] All of these concepts were objects of the present proposal, including a noninvasive approach for dead-tissue debridement as well as a new kind of transparent film with intelligent properties for treatment of large burns.

THE NEW PARADIGM: THE NEXT GENERATION OF BURNS TREATMENT

We propose that the extensively burned patient should be completely treated as soon as possible after admission to the hospital, which means in the first day or days, removing as soon as possible the dead tissue and restoring the skin barrier preferably in only a single procedure to prevent dehydration, loss of body temperature regulation, and invasion of microorganisms that can cause infections, often leading to sepsis, multiorgan failure, and death, as well as late functional and esthetic defects. The novel proposed treatment should be a combination of controlled enzymatic scarectomy; intelligent transparent polymeric films, acellular intelligent matrices, and MSCs and/or ERCs.

The objective of our effort was thus to develop a biocompatible NPF system as well as a general strategy for wound healing by programmatic release of bioactive agents, designed orientation of cells by selective adhesive interactions, and control of critical physical parameters (e.g., moisture, temperature) at the wound microenvironment.

Controlled Enzymatic Debridement

For many years our group and others have been working on the use of papaya and other naturally derived enzymatic formulations for scarectomy of deep burns.[10] Traditional surgical scarectomy procedures cannot precisely identify the limits between living and dead tissues. In this way, important amounts of still vital matrix are generally lost. Enzymatic scarolysis easily eliminates dead debris while preserving living tissues, which is extremely important at the time of resurfacing the patient, wherein a common situation is a lack of substituting tissue, especially dermis, and the need to minimize the esthetic and functional sequelae.[11] These debriding enzymes can be encapsulated in biodegradable NPFs with programmable release activation in the wound by heat emission from another kind of NPF with magnetite. Temperature is one of the most fundamental parameters to consider in enzyme activation and controlled scarectomy.

Intelligent Films (IFs)

Polymeric films have been extensively used around the world for the repair and closure of wounds. The basic principle is to cover the wound with a semipermeable material creating an accelerated healing environment while avoiding dehydration, infection, and trauma over the injury and allowing regular inspection by using transparent materials.[12] For >15 years, we have been using a simple transparent film made of resinite, like that used for microwave cooking but sterilized at the Burns Hospital of Buenos Aires City, Argentina, to treat burned patients. Film utility can also be of great significance in enzymatic debridement of large burns, ulcers, and other skin lesions. The ideal film should be multifunctional enough to change from an occlusive nonbreathable state, to preferably a breathable one capable of allowing water transit. Because a more breathable film is necessary, it looks more like a perforated sheet. The thickness of this film should also be variable as necessary. Such a wound dressing can be obtained by providing a wound-contacting polymeric film with various biodegradable NPFs.

Temperature control on a dressing or film is also necessary for the control of the healing process, including the biochemical debridement phase, in which some enzymes can be activated by heat. Our experiments have shown that iron nanoparticle suspensions (with a magnetic core of the size of tens of nanometers), coated with a polymer, can efficient dissipate electromagnetic energy (radiofrequency [RF] range 100 kHz–1 MHz) as heat. This needed heat can be generated by RF radiation provided by an external unit that allows for heating of iron oxide–coated NPFs distributed over the polymeric films.[13,14] By reaching temperatures of 37°C–45°C, various enzyme preparations, such as papain loaded in biodegradable NPFs, can be activated under these films to begin and to control wound debridement. In the same way, temperature can induce these films to open their pores and change water vapor transport as desired. Furthermore, pH can be modified as needed to improve or retard biodegradability of these NPFs. RF energy can be given without affecting the patient's overall health.

Use of Mesenchymal Stem Cells in Burn Wounds

MSCs have been used in many experimental and clinical studies demonstrating their safety and efficacy. Also, there

is quite a lot of experience in their use in various wound models as well as in burns. In 2003, porcine bone marrow–derived MSCs (BM-MSCs) in fibrin glue were grafted into the skin wound of a minipig. The cells differentiated into vascular endothelial tissue, forming new blood vessels and thus improving wound healing.[15] Likewise, 40 Wistar rats with deep burn wounds were transplanted with allogeneic and autologous BM-MSCs resulting in the formation of new blood vessels and granulation tissue with a rapid decrease in the burn area.[7] In 2004, 72 deep–partial-thickness burn wounds on the backs of six minipigs were treated with BM-MSCs autografted onto the skin wounds. There was a decrease in the wound with complete healing by day 21 after the injury.[16] In 2005 we applied human MSCs locally and intravenously in mice with skin and spinal cord injuries, observing improved healing and no immune rejection.[17]

MSCs have also been used to treat radiation-induced complications, especially those caused by radiotherapy. Intravenously infused MSCs in irradiated mice (30 Gy locally on the thigh) reduced the extent of damage and the healing time.[18] In 2006, BM-MSCs from Wistar rats were applied to injured skin; after 7 days, the MSCs differentiated into sebaceous glands and hair follicles expressing proteins of those tissues, demonstrating that MSCs can differentiate into cells of skin appendages.[19] In 2006, MSCs from human healthy donors were transplanted into rat corneae bearing chemical burns. MSCs were able to reconstruct the rat corneal surface with differentiation into corneal epithelial cells.[20]

In 2005 the first human study with MSCs was conducted. A female patient in Russia with extensive burns (I-II-IIIAB skin burn, total area 40%, area of IIIB degree 30%) was transplanted with allogenic BM-MSCs; as a result of the angiogenesis stimulated by the MSCs, she displayed rapid healing of the wounds and faster rehabilitation.[21] In 2007, a young victim from Chile bearing a severe radiation burn was treated in France, with a combination of dosimetry-guided surgery and local application of autologous MSCs. No adverse events were reported, there was no recurrence of radiation inflammation or radiation pain, and the wound rapidly started to heal.[22] Osiris Therapeutics, with funding from the USA Department of Defense, produced Prochymal in 2007; it is an intravenous infusion of BM-MSCs for the treatment of acute radiation syndrome and its consequences, such as cutaneous radiation syndrome (radiation burn). Currently, the product is in FDA clinical trial phase III.[23] In 2006, a patient bearing a diabetic foot was treated with a combination of autologous BM-MSCs and autologous fibroblasts on a biodegradable collagen membrane (Coladerm); MSCs were applied to the wound twice via injection into its edges. The outcome was a steady decrease in wound size and increase in vascularity.[24] In 2007, patients with acute wounds from skin cancer surgery and those with chronic lower extremity lesions were treated with topical application of autologous BM-MSC using a fibrin polymer spray system. There were no adverse events, and there was a major decrease in wound size.[25] In 2007, 18 of 20 patients with nonhealing chronic dermatopathies who were treated with an artificial dermis composed of collagen sponge seeded with autologous MSCs improved significantly.[26]

Endometrial Stem Cells for Wound and Burn Treatment

ERCs are a particular type of stem cells that are found in the menstrual blood of young women. They have been fully characterized, and their karyotype is stable after several passages. They demonstrate interesting regenerative capacities, especially in ischemic sites, where they are supposed to generate a great angiogenic stimulus.[27] These cells appear extremely interesting for use in the treatment of large burns, but especially skin lesions where vascularization has deteriorated, as in chronic diabetic ulcers. Therefore, our plans for the future in the development of new skin tissue engineering products with this type of cell or in combination with MSCs.

Intelligent Acellular Matrices

Since the early 1990s, we believed that pig skin could produce not only transient but also permanent coverage for burn wounds, especially when its dermis is used after rendering it acellular. In this way, we developed a program to produce a dermal matrix of pig origin. Since then, more than 10,000 m^2 of this biologic material sterilized by gamma irradiation has been applied to our burns patients, obtaining permanent integration of the scaffold, reduction of morbidity and mortality, and significantly fewer functional and esthetically unappealing chronic defects. These matrices are easy and cheap to produce; there are no availability problems such as those observed with cadaveric human skin. They rapidly integrate into the wound, providing a three-dimensional scaffold with the same chemical, physical, and electrical characteristics as the original human dermis, allowing for stem cell homing and differentiation.[28]

This technology was a substantial advance in wound treatment of burned patients when used under the proper transparent resinite films. Making these acellular pig matrices intelligent by providing them with biodegradable NPFs coated with special monoclonal antibodies, such as anti-CD44, and loaded with specific growth factors, antibiotics, and/or cytokines allows them to be rapidly populated by homing of atologous bloodstream MSCs which differentiate in the most friendly environment, also under the protection of an intelligent transparent film. These “intelligent matrices” can be designed to generate “specific signals for stem cell homing and adhesion,” to produce “specific alarm signals for stem cell mobilization and homing,” or to have the ability “to attract and fix MSCs to its specific epitopes, such as anti-CD44” as well as provide a structured matrix for improved wound healing.

We showed for the first time that MSCs circulate in large amounts in the bloodstream of patients with large burns.[29] The proposed biomaterial encapsulation technology for

NPF production possesses several clear advantages over conventional encapsulation strategies. First, it can encapsulate combinations of delicate biomaterials, such as proteins, under conditions of little or no mechanical stress (very low shear). Second, it provides hollow submicron structures of a wide variety of materials, which is a properly that virtually all capsule-forming approaches lack. Third, the hollow fiber monodispersity is easy to achieve with controllable sizes from 7 to 30 μm. Fourth, it offers shell thickness control, which is normally as thin as 20–30 nm, as well as shell porosity control. Fifth, shell formation occurs by mere evaporation of the shell solvent (e.g., water or ethanol-water mixtures) during the time-of-flight of the droplet-in-droplet electrospinning assemblies from nozzle to collector, i.e. it is not a process involving chemical reactions. Sixth, the ease of fabrication, is transferable to a wide range of drug encapsulation needs. Thus, local release of biomaterials and growth factors as well as the inclusion of an anti-CD44 monoclonal antibody in some NPFs with the ability to attract and orient critical cells involved in the wound healing repair process can significantly increase the survival chances of burn patients, minimizing side effects associated with infection and dehydration with current treatment modalities. In addition, the formulations of the skin tissue scaffolds in the present proposal naturally reabsorb into the human body in a specified time while the healing process progresses and contributes to the generation of new necessary cellular elements and de novo extracellular matrix for gradual and esthetically acceptable skin replacement. Furthermore, these original skin tissue scaffolds proposed herein have one added advantage: When made in this form with NPF deposits in broad fiber diameter distributions, or fibers or bead-fibers combined with particles, the bioactive agents that aid in the healing process are released in a sustained programmable manner. This is due to the fact that various diameters, polymer thicknesses, and/or characteristic lengths of NPFs translate into distinct release rates of the entrapped bioactive agent/agents from the scaffold material, depending on the characteristic length of the NPFs.

It is also envisioned that the three-dimensional conformational, biochemical, and electrical configuration of these natural acellular matrices not only from dermis but also from any other human or animal organ will be able to be used in the construction of synthetic scaffolds with biodegradable biopolymers.[28] In this case, our technology based on nanoengineered multifunctional NPFs, especially those coated with anti-CD44 as well as with other monoclonal antibodies or substances that are important for the homing, adhesion, proliferation, and differentiation of circulating or delivered stem cells, will also be of great utility to facilitate regeneration in various organs and tissues.

The Paradigm in the First Clinical Trial

With this approach in mind we are designing the first clinical trial for the treatment of extensive full-thickness burns with MSCs. In a second trial we will try to use ERCs alone and in combination with MSCs, hoping to see more rapid healing due to improved neoangiogenesis. The latter approach will probably be most valuable in skin ulcers, such as those observed in diabetic patients.

DISCUSSION

Our nanoengineered multifunctional matrices, wound dressings, and films greatly advance our technical ability to selectively deliver and release shielded biomaterial into a wound as well as to selectively anchor and adhere proper repair cells. As has been said above, we are using multicomponent multifunctional core-shell magnetic NPFs for thermal control or monoclonal coated nanoparticles, depending on their use for the fabrication of films or matrices, respectively. In addition, delivery of BM-MSCs obtained from living or cadaver donors into the wound, the airway, or intravenously will probably improve the healing process and the inflammatory reactions that are observed in all burned patients, including those suffering from a radioactive origin. ERCs will surely be a plus to be introduced in the bottom of these constructs, especially in lesions of ischemic origin, by improving neoangiogenesis and vascularization of skin substitutes. Our method will surely address not only the burns problem but also other serious chronic nonhealing wounds, such those observed in diabetic patients. Finally, we are convinced that this technology will have great potential in the entire tissue and organ regeneration field.

REFERENCES

1. Gomez M, Wong DT, Stewart TE, Redelmeier DA, Fish JS: The FLAMES score accurately predicts mortality risk in burn patients. J Trauma 65:636, 2008

2. Samadikuchaksaraei A: Engineering of skin substitutes: current methods and products. In Greco GN, ed. Tissue Engineering Research Trends. Hauppauge (NY): Nova Science; 2008, p251–66.

3. Atiyeh BS, Costagliola M: Cultured epithelial autograft (CEA) in burn treatment: three decades later. Burns 33:405, 2007

4. Bannasch H, Unterberg T, Föhn M, Weyand B, Horch RE, Stark GB: Burns: cultured keratinocytes in fibrin with decellularised dermis close porcine full-thickness wounds in a single step. Burns 34:1015, 2008

5. Bannasch H, Stark GB, Knam F, Horch RE, Föhn M: Decellularized dermis in combination with cultivated keratinocytes in a short- and long-term animal experimental investigation. J Eur Acad Dermatol Venereol 22:41, 2008

6. Kopp J, Jeschke MG, Bach AD, Kneser U, Horch RE: Applied tissue engineering in the closure of severe burns and chronic wounds using cultured human autologous keratinocytes in a natural fibrin matrix. Cell Tissue Bank 5:89, 2004

7. Shumakov VI, Onishchenko NA, Rasulov MF, Krasheninnikov ME, Zaidenov VA: Mesenchymal bone marrow stem cells more effectively stimulate regeneration of deep burn wounds than embryonic fibroblasts. Bull Exp Biol Med 136:192, 2003

8. Laschke MW, Harder Y, Amon M, et al: Angiogenesis in tissue engineering: breathing life into constructed tissue substitutes. Tissue Eng 12:2093, 2006

9. Murphy MP, Wang H, Patel AN, et al: Allogeneic endometrial regenerative cells: an "Off the shelf solution" for critical limb ischemia? J Transl Med 19:45, 2008

10. Smith RG: Enzymatic debriding agents: an evaluation of the medical literature. Ostomy Wound Manage 54:16, 2008

11. Weir D, Farley KLJ: Relative delivery efficiency and convenience of spray and ointment formulations of papain/urea/chlorophyllin enzymatic wound therapies. Wound Ostomy Continence Nurs 33:482, 2006

12. Chaby G, Senet P, Vaneau M, et al: Dressings for acute and chronic wounds: a systematic review. Arch Dermatol 143:1297, 2007

13. Shinkai M, Ueda K, Ohtsu S, Honda H, Kohri K, Kobayashi T: Effect of functional magnetic particles on radiofrequency capacitive heating. Jpn J Cancer Res 90:699, 1999

14. Shinkai M, Ueda K, Ohtsu S, Honda H, Kohri K, Kobayashi T. Effect of functional magnetic particles on radiofrequency capacitive heating: an in vivo study. Jpn J Cancer Res 93:103, 2002

15. Fang LJ, Fu XB, Sun TZ, et al: An experimental study on the differentiation of bone marrow mesenchymal stem cells into vascular endothelial cells. Zhonghua Shao Shang Za Zhi 19:22, 2003

16. Fu XB, Fang LJ, Wang YX, Sun TZ, Cheng B: Enhancing the repair quality of skin injury on porcine after autografting with the bone marrow mesenchymal stem cells. Zhonghua Yi Xue Za Zhi 84:920, 2004

17. Mansilla E, Marin GH, Sturla F, et al: Human mesenchymal stem cells are tolerized by mice and improve skin and spinal cord injuries. Transplant Proc 37:292, 2005

18. Bensidhoum M, Gobin S, Chapel A, et al: Therapeutic effect of human mesenchymal stem cells in skin after radiation damage. J Soc Biol 199:337, 2005

19. Fu X, Qu Z, Sheng Z: Potentiality of mesenchymal stem cells in regeneration of sweat glands. J Surg Res 136:204, 2006

20. Ma Y, Xu Y, Xiao Z, et al: Reconstruction of chemically burned rat corneal surface by bone marrow–derived human mesenchymal stem cells. Stem Cells 24:315, 2006

21. Rasulov MF, Vasilchenkov AV, Onishchenko NA, et al: First experience of the use of bone marrow mesenchymal stem cells for the treatment of a patient with deep skin burns. Bull Exp Biol Med 139:141, 2005

22. Lataillade JJ, Doucet C, Bey E, et al: New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy. Regen Med 2:785, 2007

23. Fact sheet: Prochymal therapy as a medical countermeasure for acute radiation syndrome. Osiris Therapeutics, Inc. Revision. Available at: www.osiris.com

24. Vojtassák J, Danisovic L, Kubes M, et al: Autologous biograft and mesenchymal stem cells in treatment of the diabetic foot. Neuro Endocrinol Lett 27(Suppl 2):134, 2006

25. Falanga V, Iwamoto S, Chartier M, et al: Autologous bone marrow–derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng 13:1299, 2007

26. Wu Y, Chen L, Scott PG: Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 25:2648, 2007

27. Murphy MP, Wang H, Patel AN, et al: Allogeneic endometrial regenerative cells: an "off the shelf solution" for critical limb ischemia? J Transl Med 19:45, 2008

28. Mansilla E, Drago H, Sturla F, et al: Matrix superhighways configurations: new concepts for complex organ regeneration. Transplant Proc 39:2431, 2007

29. Mansilla E, Marín GH, Drago H, et al: Bloodstream cells phenotypically identical to human mesenchymal bone marrow stem cells circulate in large amounts under the influence of acute large skin damage: new evidence for their use in regenerative medicine. Transplant Proc 38:967, 2006