The place of tissue engineering in wound repair
The 3 “R”s: Repair, Replace, Regenerate

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Skin has a considerable potential for efficient and functional repair. However, while cutaneous repair is a regenerative process in the foetus, this capability declines in late gestation as inflammation and scarring alter the outcome of healing. Scar tissue lacks a normal extracellular matrix (ECM) organisation and the epidermis covering the scar fails to develop appendages such that postnatal wound healing substitutes repair for regeneration.1

Skin, the largest organ of vertebrates (±10% of the body mass), is critical to defence and survival. Loss of its integrity may result acutely in substantial physiologic imbalance and ultimately in disability or even death. Thermal injury as well as chronic ulcerations secondary to diabetes mellitus, pressure and venous stasis are the primary causes of significant skin loss in man2 and represent a major impetus for research into tissue engineering. In veterinary medicine we likewise struggle with the number and complexity of wounds our patients suffer. Specifically, horses respond to danger with a fight-or-flight instinct, predisposing them to massive skin wounds that are not amenable to primary closure but which must heal by second intention. In these cases, problems in the wound repair process are frequent and may lead to the development of either a chronic non-healing wound or to extensive scarring which adversely affect function, often through lameness.

To that effect it is reported that roughly 7% of injuries leading to the retirement of race horses in New Zealand are the direct result of a wound.3 If that data is extrapolated to the European situation, where roughly 1 million horses are involved in athletic competition, one might expect that the annual withdrawal of 70,000 horses from competition would bear a significant financial impact on the equine industry.

The historical gold standard for replacement of lost skin is the autologous skin graft. However, the horse’s lack of redundant donor skin limits the practicality of full-thickness grafting to relatively small wounds. Alternatively, allografts of cadaver skin can be used as temporary cover but are subject to rejection because the presence of antigens may elicit an immune reaction in the recipient. In fact, this type of biologic dressing was shown to offer no advantage over a nonbiologic dressing for treatment of skin wounds in horses.4

The aim of tissue engineering is to design and construct, in the laboratory, a living component that can be used for the maintenance, repair or replacement of malfunctioning or lost tissues.5 In the case of skin, the ultimate goal is to rapidly generate a construct that effects the complete regeneration of functional skin, including all its layers and appendages, as well as an operational vascular and nervous network, with scar-free integration within the surrounding host tissue. In addition to being available “off the shelf”, such a construct should enable the skin to fulfill its usual functions: barrier formation; defence against UV irradiation; thermoregulation; and mechanical support and protection. Two major approaches are used to engineer replacement tissue: in vivo and in vitro.

The in vivo approach attempts to create an acellular biomaterial that contains clues conducive for recruitment of host cells into the biomaterial and inductive to stimulate invading cells to proliferate, synthesize new ECM and, if required, differentiate in an effort to achieve a regenerative rather than a reparative wound environment. Acellular tissue-engineered constructs rely on biopolymers to provide mechanical support for tissue in-growth, and on biomimetics to induce key cell functions. Materials used as artificial ECM include those derived from naturally occurring materials (such as hyaluronan, glycosaminoglycans, fibronectin, collagen, chitosan and alginates), which have low toxicity and induce only a mild chronic inflammatory response, and those manufactured synthetically (such as polyglycolide, polylactide, polylactide-coglycolide, polytetrafluoroethylene and polyethylene terephthalate). Matrices most used in therapeutic applications are made from polymers that are often resorbed or degraded by the body. The two key challenges facing engineers are the deficiencies presented by the biopolymers in terms of mechanical and degradation properties and the difficulty in converting these polymers into scaffolds that have defined shapes and a suitable porous internal architecture that can direct appropriate tissue growth. A number of products are commercially available for veterinary use though only a few have been evaluated in dermal wounds in a controlled fashion.6,7

In contrast, the in vitro method of developing engineered tissue attempts to create organs, in tissue culture or bioreactors, for implantation and replacement of diseased or damaged tissue. Skin replacement templates arguably represent the first and most clinically successful “tissue engineering” solution designed for organ reconstruction.7 Conventionally, the approach has been to create a very simple skin substitute: disaggregated autologous (from small punch biopsies) or allogenic (from cadavers or from neonatal foreskin) keratinocytes are
grown in vitro over a period of two to three weeks and subsequently seeded onto a scaffold to form an epidermal sheet which is then placed within the wound. However, in the past years, the biotechnological approach to skin replacement has evolved from this simple cultured autologous epidermal sheet to more complex bilayered cutaneous substitutes wherein keratinocytes are co-cultured with a dermal substitute and exposed to the air interface to create a stratified epithelium. The fibroblasts forming the dermal substitute slowly degrade the biopolymer and lay down their own ECM which contributes to the formation of a basement membrane. As cell numbers increase, there is a concomitant rise in the concentration of soluble mediators (cytokines and growth factors) released into the microenvironment to exert a direct effect on epidermal proliferation, differentiation as well as formation of ECM. Nevertheless, these bioengineered bilayered cutaneous substitutes, of which a number are commercially available for clinical use in human medicine, function only as a biological dressing since they lack a vascular plexus to supply nourishment to the grafted epidermis. Moreover, the absence of differentiated structures translates into lack of temperature control provided in normal skin by sweat and sebaceous glands, as well as hair follicles. Additionally, in presently available substitutes, insulation and an adequate vascular supply from adipose tissue do not exist. Skin substitutes do not have a nerve supply, precluding sensation both of temperature and pressure. Finally, skin substitutes have no resident Langerhans cells which play an important function in immune regulation in the skin. In an effort to address some of these deficiencies, researchers have sought to develop an endothelialized reconstructed skin substitute by combining keratinocytes, fibroblasts and endothelial cells in a collagen sponge, which markedly increases the speed of vascularity by inoculation of the construct’s capillary network with the host’s vasculature.

Since at the present time there are no models of an artificial (replacement) skin that completely replicate the normal uninjured organ, a parallel area of tissue engineering has focused on true regeneration. The epidermis is a rapidly self-renewing tissue which maintains homeostasis by constant proliferation of the basal layer of rapidly dividing progeny of stem cells. Epidermal stem cells reside in specific niches located in the bulge region of the hair follicle and, upon division, produce new stem cells as well as daughter cells that enter a differentiation pathway thus joining a mature non-dividing population. Epidermal stem cells, K19-positive, slow cycling and highly proliferative, are one type of adult tissue stem cell (adult stem cells located outside the bone marrow) and are considered multipotent, that is, more restricted in their potential and functions than are totipotent embryonic stem cells (ESC) originating from the blastocyst. Unlike ESCs, epidermal stem cells are incapable of differentiating into all cell types characteristic of the species from which they derive, rather, they represent the body’s natural source of cells committed to skin homeostasis and repair. Other adult mesenchymal stem cells (MSC), notably of blood or bone marrow origin, might also contribute to regeneration of the complex organ that is skin, via their characteristic plasticity which enables a change in stem cell differentiation from one cell type to another. For example, Badiavas et al. (2003) showed that wounding stimulated engraftment of exogenously administered bone marrow-derived cells to incorporate and differentiate into non-hematopoietic skin structures. While no reports have been published on their use in skin wounds in the horse, equine MSCs of both adipose and bone marrow origin are proposed for the treatment of tendon and ligament injuries and have been shown in an experimental model of articular cartilage defects to improve the early healing response following their arthroscopic implantation.

The challenge in regeneration research is to understand how the events of tissue injury, which would normally lead to scarring, can be coaxed towards the activation of plasticity in residual progenitor cells, enabling tissue regeneration. One solution might be to identify the molecules expressed during regeneration and incorporate them into a “smart matrix” for use in the creation of a skin equivalent. This will require isolation and characterisation of genes and proteins involved in both repair and regeneration. With this aim in mind, we recently began constructing a comprehensive database of gene expression during healing and non-healing in the horse. Our first study identified a number of genes that had not previously been attributed a role during wound repair, in any species. These novel genes, up-regulated during the proliferative phase of repair of body wounds in horses might be differentially expressed in limb wounds compared to body wounds; in horses compared to ponies; or in wounds becoming chronically inflamed or developing exuberant granulation tissue compared to those healing normally. We subsequently cloned full-length cDNAs of selected genes in view of correlating changes in gene expression with spatio-temporal protein expression within repair tissues.

In conclusion, the ultimate tissue engineering goal in the realm of dermal wound repair would be to combine the various stem cell types with or without a natural or synthetic matrix in the presence of molecules known to promote regeneration.

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1. Vet-Stem (Poway, CA, USA).
REFERENCES