Horses can be subject to traumatic wounds for which the healing process is often complicated and slow compared to that in ponies and other related species. Problems are usually most severe on the lower limbs (Wilmink et al., 2005), with primary closure (suturing) being the preferred method of dealing with such injuries as it both reduces the time taken to heal and improves the cosmetic appearance of the final scar. In cases where there is unmanageable contamination, excessive compromise or loss of tissues, or where there is a risk that the wound may burst open following suturing, however, many of these wounds are left open for new skin to form naturally. Unfortunately, the state of such open wounds is often made worse by their propensity to develop exuberant granulation tissue, seen as proud flesh. Structurally, this proud flesh is connective tissue, blood vessels and fibroblasts, and it can be extremely slow to heal, is liable to become infected and can completely inhibit the formation of new epidermis. It can leave horses lame and permanently scarred, resulting in a substantial decrease in the economic value of the animal, particularly for show and performance horses. Not surprisingly then, there are many products available on the market that purport to promote wound healing, though the scientific validity of many of the claims made is often lacking. Furthermore, claims for new plant extracts with wound healing properties appear regularly. Identifying and assessing the validity of such compounds for the development of effective wound treatments is therefore an important research objective for scientists in IBERS.

While experimentation in the field of wound healing has traditionally been carried out on live animals, this presents a number of very substantial problems. Naturally occurring wounds are highly diverse in terms of both their development and physiology, so very high numbers of accidental wounds would be required to make experiments statistically valid. Obtaining access to a statistically valid population of wounded animals would require the recruitment of large numbers of veterinary surgeons and horse owners for clinical trials. Such procedures are far too expensive to run other than for a product that is almost ready for the market and would certainly not be appropriate for a compound for which there is little good evidence of efficacy. The alternative of creating experimental wounds in a managed population of live animals with ready access raises a number of significant ethical questions. Wounding healthy animals is of dubious ethical status, and the wounding of horses is very unlikely to receive approval from the Home Office Animals Scientific Procedures Division in the UK. Furthermore, leaving a control group untreated, as the traditional scientific method demands, would compound the unethical nature of the research. Besides the ethical aspects, it is also difficult to investigate individual stages of the healing process in the live animal due to the complicated interactions between the different stages of recovery and the varying behaviours of individual animals. This type of experimentation has also been shown to be lengthy, laborious and expensive (Sullivan et al., 2001).

More recently, however, there has been a growing interest in the use of laboratory in vitro models to investigate the mechanics of wound healing (Houghton et al., 2005). An accurate model of wound processes in vitro would enable precise control of the ‘wound’ environment to be established; would allow much speedier and cheaper investigations to be performed and would avert the ethical implications of experiments on live animals (Gottrup et al., 2000). Additionally, an in vitro wound environment is considerably less variable than that in a live animal, however caused. More importantly, laboratory models would also facilitate
the high throughput screening of potential treatments, with mode of action being determined at a cellular level (Tiffany-Castiglioni et al., 1999). Nevertheless, there are a number of limitations to this in vitro approach. Many models have only one cell type present; some do not include any systemic hormonal or immune organisation, and the simplistic construction of the cell microenvironment can make extrapolation of results to the live animal very difficult (Freshney, 2000).

At IBERS, we have developed two in vitro assays that attempt to model the wound healing process using equine fibroblasts. In horses, the rate at which a wound contracts following injury directly affects the likelihood of effective wound healing. Wound contraction is brought about by the fibroblast cells differentiating into myofibroblasts, specialised cells with muscle-like properties which are able to bring the edges of a wound together. These myofibroblasts construct and then continually rearrange a surrounding matrix of collagen tissue while contracting, thereby permanently leaving the skin in its new position. The ability of fibroblasts to reconstruct skin can be investigated by dispersing them into collagen gels and observing the extent of gel contraction (Carlson & Longaker, 2004). This is a well established method and one that we have been using to identify the effect of various plant extracts on myofibroblast contraction in vitro. A schematic of this procedure is included as Figure 8.1. The actual contraction of an experimental gel containing dispersed fibroblasts is shown in Figure 8.2.

Cell migration is thus a critical part of the wound healing process. To study this more closely, we grew fibroblasts in a monolayer directly on a plastic surface designed for tissue culture and then cleared a known area of these cells using a vacuum. Adjacent cells around the edge of the clearing then repopulate the cell-free area through the combined action of migration and cell division (Calderon et al., 1996). The progression of this cellular re-population on the monolayer surface can be examined by microscopy and image analysis, as illustrated in Figure 8.3. Pre-treating the fibroblast monolayer with a DNA synthesis inhibitor such as Mytomycin C restricts the repopulation of the cleared area of cells to cell migration processes, since cell division will have been inhibited. The impact of cell proliferation in this model can therefore be determined by the difference between runs with and without the DNA synthesis inhibitor.

As an example of the natural products which have been shown or are claimed to promote wound healing, Aloe vera gel, extracted from the central zone of the Aloe vera leaf, is thought to contain approximately 75 potentially active components (Atherton, 1998). In humans, burn wounds are treated with Aloe vera in a number of countries (Marshall, 1990; Reynolds & Dweck, 1999), and various studies have plausibly suggested that it promotes wound healing (e.g., Chithra et al., 1998). Modes of action are thought to include the penetration and anaesthesia of tissue, inhibition of bacterial and fungal growth, anti-inflammatory effects and the dilation of capillaries to encourage blood flow. In terms of wound contraction, Aloe vera gel is thought to enhance the strength of the myofibroblasts (Heggers et al., 1996). Plants for which wound healing evidence is much weaker include marigold (Calendula officinalis), comfrey (Symphytum officinale) and knotted figwort (Scrophularia nodosa), amongst others. All of these plants, together with a range of further potential candidates and control species, have been grown by scientists in IBERS under hydroponic conditions. Using a variety of techniques, potentially active...
components have then been isolated from their leaves, roots and flowers for inclusion in the in vitro assays. While our findings are still very much at a preliminary stage, early results have suggested that some extracts of Aloe vera, comfrey and marigold are able to promote the wound contraction process using fibroblasts extracted from equine tissue. Research is now continuing to identify more precisely those particular extracts which may be able to promote wound healing, to isolate their active ingredients, and ultimately to develop novel sources for successful treatments.

References


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Figure 8.3. A. A confluent lawn of fibroblast cells cultured on plastic 0 hours after the removal of a circular area of cells, B the same area of cells after 24 hours, and C the same area after 48 hours.