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Abbreviations and Acronyms

α -SMA = alpha-smooth muscle
actin

CCAC = Canadian Council for
Animal Care

Hsp-70 = heat shock protein 70

Krox-24 = early growth
response 1

MRL/MPJ mouse = Murphy
Roths Large derived by the
Murphy group of the Jackson
Laboratory

Msx-1 = muscle segment
homeobox 1

p53 = protein 53

TGF- β = transforming growth
factor beta

Axolotl as a Model to Study Scarless Wound Healing in Vertebrates: Role of the Transforming Growth Factor Beta Signaling Pathway

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Significance: The skin is our largest organ, with the primary role of protection against assaults from the outside world. It also suffers frequent damage, from minor scrapes to, more rarely, complete destruction such as in third-degree burns. It is therefore, by its nature, an organ that would benefit tremendously from being able to regenerate itself.

Recent Advances: This review highlights the axolotl, a less well-known model organism capable of scarless wound healing and regeneration. Axolotls are salamanders with unsurpassed healing and regenerative capacities. Understanding how these animals can regenerate their tissues could help identify the pathways that need to be activated or inhibited in humans to improve wound healing.

Critical Issues: Presently, there are no therapies leading to skin regeneration or scarless wound healing. Various animal models have thus been developed for use in research, such as mice and pigs, to help us understand how wound healing could be improved or stimulated. However, these more common models cannot regenerate and, consequently, cannot direct us toward a solution to regenerate damaged tissues. Axolotls, on the other hand, can regenerate perfectly and therefore may offer avenues to identify molecular targets for therapeutic intervention.

Future Directions: Identifying signaling pathways regulating tissue regeneration in vertebrate models is important. The use of animals such as axolotls, which hold the secret of full regeneration, will likely play a significant role in helping us achieve scarless wound healing for humans.

SCOPE AND SIGNIFICANCE

THIS CRITICAL REVIEW looks at the advantages and disadvantages of different experimental animal models used to study wound healing and skin repair. Humans and mammals, in general, heal their skin wounds by scarring when the dermis is damaged. Clinically speaking, it would be much better to regenerate damaged skin as opposed to scarring. Scar

tissue has lower tensile strength and is less flexible. We present the axolotl (Mexican salamander) as a model organism to study scarless wound healing in vertebrates. These animals, which are urodele amphibians, are the only vertebrates that can regenerate/heal their tissues perfectly throughout their lives. This is important as these animals offer a unique opportunity to identify the

mechanisms and signals that allow animals to heal perfectly.

TRANSLATIONAL RELEVANCE

A better understanding of how different vertebrates heal their wounds is essential to identify the pathways that are (1) responsible for scarring and (2) responsible for regeneration. The use of experimental animal models allows the identification of molecular signals and cellular events that lead to scar tissue formation in some animals and tissue regeneration in others. The evolutionary conservation of gene function across species makes it possible to transfer the knowledge gained from regenerating to nonregenerating animals.

CLINICAL RELEVANCE

Presently, there are no treatments that lead to scarless skin wound healing. If we are to one day improve the clinical outcome for patients, we have to understand the biology behind tissue repair and regeneration. Although it cannot be said when exactly the results of basic research will provide the information/knowledge to regenerate patient skin, it is nonetheless essential to keep on doing research to identify the genes and signaling pathways that could be harnessed for therapeutic applications in the future.

INTRODUCTION

Although skin is one of our largest organs and our single most important protective layer against bacterial infection, it is often viewed and considered in terms of aesthetics. It goes without saying that the skin is responsible in large part for our looks, but it is also important for thermoregulation through perspiration. Since the skin represents our protective layer against the outside world, it comes as no surprise that it is the first and most frequent organ to be damaged or injured during our lifespan. Damages incurred are from minor scrapes to deep wounds, or in some situations, large sections being destroyed. It is therefore, by its nature, the organ that would benefit most from being able to regenerate itself. Most people would wish to be able to regenerate their skin because of its aesthetic role. Very few people cherish having a large visible scar on their skin, as opposed to scars on internal organs for which most people are happy to recover the functional aspect. Good examples are bypass surgery or bone implants (*e.g.*, hip replacement). However, being able to regenerate damaged skin is more than simply aesthetics. Scar

tissue has lower tensile strength, may lack pigmentation as well as skin appendages such as sebaceous glands, is less flexible, and sensation is often missing altogether from the damaged skin.¹ In addition, with the increasing aging population in the Western world, more cases of skin diseases are diagnosed, and more surgeries are performed for various ailments. The number of surgeries worldwide are in the hundreds of millions each year and will likely remain this high for many years to come. A large part of today's emerging older population comprises the baby boomers that are highly active and conscious of their appearance. This is in part driving the global sales figure for cosmetics, which were around \$253 billion in 2006, with growth projections of over 4% per year for the foreseeable future.² Most cosmetics are for skin care that highlights the importance of healthy-looking skin for many people worldwide. The industry is working hard to provide an array of products to help keep our skin looking healthy (or younger and less wrinkly). However, there are no products yet available that can eliminate scars or stimulate our skin to regenerate (although more and more companies are working on developing products for this purpose). This is important notably for patients with injuries such as third-degree burns on large parts of their bodies, or after surgeries for ailments such as skin cancer. Therefore, there is an important clinical need to find ways to reduce scarring and to stimulate skin regeneration.

EXPERIMENTAL MODELS

There is a significant effort by scientists worldwide to understand why human skin heals by scarring. For obvious ethics reasons, research cannot be performed directly on humans for solving the basic mechanisms on how wound healing leads to the aberrant collagen deposits observed in scars at the biochemical and cellular levels.^{1,3,4} Therefore, we turn our attention to the various experimental models that can help answer fundamental questions on how wound healing proceeds. Answering the aforementioned question is rather complicated by the fact that different types of injuries heal differently. For instance, a deep stab wound does not heal in quite the same way a superficial scrape does. There are also excisional and nonexcisional wounds. For example, the incision site for an operation is nonexcisional, whereas removing a mole or a melanoma requires taking a biopsy, which results in an excisional type of wound. The cellular dynamics required to heal an incisional wound does not require cells at the edge to migrate over the surface to

close the wound, as opposed to an excisional wound where cells have to migrate to start the healing process. Many studies on wound healing are looking at injuries resulting from a physical trauma (cut, scrape, or biopsy). However, some wounds can be the result of chemical exposure or extreme stress such as heat in burn victims. Second-degree burns, although painful, heal rather well as opposed to third-degree burns that leave the patients with dysfunctional skin, which consists of a scar devoid of the glands necessary for thermoregulation (sweat glands and sebaceous glands).¹

To increase our understanding of the cellular and molecular events that regulate wound healing, scientists have turned to various models. The *in vitro* approach of developing skin cultures and substitutes has seen much progress in the last 20 years.^{5,6} There are many interesting prospects to using artificial systems to study skin biology; however, due to space constraints and to maintain the focus on animal models, we will refer the readers to reviews on that subject by Grinnell (2003)⁶ and by Poumay and Coquette (2007).⁷ Generally, when one is thinking of wound healing, the focus is on human welfare, and since we are mammals, we often consider that the best animal models should also be mammals. There is indeed a certain phylogenetic logic to this thinking process (see Fig. 1), and it is without anyone's surprise that a large proportion of the research being done today using animals is on small mammals (*i.e.*, rodent models). According to the Canadian Council for Animal Care (CCAC),⁸ mice and rats represent nearly 40% of all the animals used in research (biomedical, agricultural, and academic research in Canada). The only other group of animals that comes close is fish, probably due to the ever-increasing use of the zebrafish, which is bred in large numbers for genetic screens. When browsing the 2010 statistics of the CCAC, one realizes quickly that small animals are highly prized for research, while large animals (bigger than a pig or sheep) represent <2.5% of the total animals used.⁸ Obviously, the high cost of housing and feeding animals precludes the vast majority of research laboratories from being able to perform research on large animals.

Mouse model

Mice represent the animal model that is most frequently encountered in laboratories for biomedical research. They are presently the only vertebrate model in which we can delete any genes, knock-in specific mutations, perform tissue-specific deletions, and control when the deletions will occur. The mouse genome was fully sequenced

> 10 years ago,^{9,10} and thousands of antibodies that recognize its proteins are commercially available, making it one of the animal models for which there is the largest tool set available to perform experiments. There are multiple mutant mouse models that have been used to study the role of genes during wound healing, and there is even a model that has been presented as an actual mammalian model that can regenerate its tissues, the MRL/MPJ mouse.¹¹ It seems that these mice have the ability to heal the punch holes made on their ears for identification, in every respect perfectly.¹¹ This new mouse strain obviously generated quite a lot of excitement. Unfortunately, when their ability to heal skin wounds was tested, it became apparent that wound healing in these mice resulted in scarring just like the wild-type littermates.¹²

So far, no mouse models have been developed that can heal skin wounds perfectly. However, a recent study has identified a close cousin of the laboratory mouse, called the spiny mouse (*Acomys kemp* and *Acomys percivali*), as being able to heal large wounds without the appearance of scars, which indicates that some mammals are capable of what seems to be skin regeneration as adults.¹³ This report is the first to show such extensive wound-healing capacities in a mammal and raises the hope that regeneration is not completely lost in this class. That same report also looked at the ability of these spiny mice to regenerate ear holes, which they were able to do very well, although not with 100% efficiency, since the muscle covering the cartilage was not regenerated.¹³ A high contraction rate of the wounds was observed in spiny mice with

Figure 1. Rooted phylogenetic tree depicting evolutionary relationships between various organisms. The invertebrates (*Caenorhabditis elegans* and *Drosophila melanogaster*) and vertebrates (all other animals in this tree) are separated. Notice that vertebrates are further subdivided in different classes: fish, amphibians, and mammals. The animals discussed in the article are underlined, and it is apparent that pigs are closest to humans, followed by mouse and then axolotls. This tree represents a partial phylogenetic analysis and is intended as an aid to help visualize the evolutionary relationship of these animals. This tree was generated with software Lasergene Meg-Align from DNASTAR using the *p53* sequences from each organism in the tree.

a 65% reduction in the wound area within 24 h.¹³ Unfortunately, the report did not test the ability of these animals to heal their skin after a second wounding event, and it did not look at whether the panniculus carnosus, which is essential for wound contraction in loose-skin animals, was regenerated at the wound sites.¹³

As in any animal model, the mouse has its limitations when comparing its skin wound-healing ability. Differences in the skin biology between mice and humans preclude the mouse from being the perfect model to mimic wound healing in humans (despite the incredible tool set available for this model). One of the problems encountered when comparing wound healing between mice and humans is the fact that mice (and spiny mice) are loose-skin animals and humans are tight skinned.^{4,14} Loose-skin animals heal mainly by wound contraction with the panniculus carnosus, which is absent in humans. Tight-skin animals, on the other hand, heal mostly by re-epithelialization over the accumulated granulation tissue. This granulation tissue arises after an inflammation phase during which various cytokines are activated, which will recruit macrophages and neutrophils in the wound bed.³ Many technical approaches were developed using ring stents, flaps, pressure, and mechanical load models to bring the mouse loose-skin model as close as possible to the human tight skin to circumvent the problems arising from loose skin and to improve its use as an experimental model system for wound-healing studies.¹⁵

Pig model

In an attempt to find an alternate solution to the problems encountered with loose-skin animals, researchers introduced the pig model. The Yorkshire pig was the first to be introduced because of the high resemblance of its skin with that of humans. Pigs are tight-skin animals that heal their skin wounds by scarring. The organization of pig skin is almost identical to that of humans.¹⁶ There is also the red Duroc pig that mimics hypertrophic scarring in humans.¹⁷ The main advantages of the pig models as compared to the mice are as follows: (1) they are tight-skinned as mentioned above; (2) they heal by re-epithelialization over the accumulated granulation tissue after an inflammation phase; and (3) their larger size allows multiple wounds to be administered on a single animal, allowing experimenters to test different types of wounds or dressings. Since the pig is phylogenetically close to humans (Fig. 1), most of the antibodies that have been raised against

human proteins cross-react with the pigs. In addition, the pig represents an interesting model in terms of pharmacokinetic studies and drug testing.^{18,19} There are of course some disadvantages of using pigs when compared to mice: the first being the cost of the animals, and second the fact that we cannot perform any genetic manipulations.

Animal models for scarless wound healing

So far, we have described animal models that aim to mimic or reproduce as close as possible the wound-healing process observed in humans, scar and all. These models are important and essential to understand what leads to scar formation. However, these models may not provide direct insight into how to achieve a scarless wound healing, which would represent a tremendous benefit to patients. Once again, researchers turn to animal models to gain insight and understanding on how scarless wound healing and/or skin regeneration can be achieved. Nature has succeeded almost always in providing models to resolve most questions for which scientists seek answers, and scarless wound healing/regeneration is no exception. Research done on mammalian fetal wound healing has shown that fetuses from some species (scarless wound healing is not a universal trait of all mammalian fetuses) can regenerate their skin if the damages occurred during the first trimester of gestation.^{20,21} This is exciting as it indicates that some mammals, other than the spiny mouse, can regenerate their skin, even though the timeline corresponds to when embryonic development is not completed and may be the result of developmental homeostasis. Another caveat is the fact that accessing embryos to perform these injuries is not trivial. The mouse uterus is very small and fragile, so accessing the fetuses is a challenge. The sheep, which is often used for fetal wound-healing studies, is of a reasonable size for accessing the fetuses, but the cost can be in many cases prohibitive. Interestingly, mammalian embryos, which can regenerate skin wounds, do so without much inflammation (immune cell infiltration and cytokine production) and seem to deposit and turnover extracellular matrix components more rapidly than adult wounds.²¹

Axolotl as a model for scarless wound healing

The availability of an animal model capable of scarless wound healing or skin regeneration at an adult stage could allow scientists to identify the cellular and molecular mechanisms regulating organogenesis in a fully developed and mature setting. Such animals do indeed exist in nature. For example, many invertebrates can regenerate

whole-body segments if cut in half (planarians and echinoderms). There is also a group of vertebrates that can regenerate whole limbs, tail, and internal organs and heal skin wounds perfectly without the appearance of any scar tissue. These animals are the urodele amphibians (*i.e.*, salamanders). The most widely used urodeles are the axolotl (also known as the Mexican salamander) and the newt. These animals can regenerate all of their appendages perfectly after amputation (Fig. 2).^{22,23} Genomic studies have consistently shown that developmentally important genes are strongly conserved across vast evolutionary distances. Much of what we know of vertebrate development and molecular genetics has been derived from studies on *Drosophila melanogaster* and *Caenorhabditis elegans*. Therefore, it is not unreasonable to expect that the same genes involved in the regeneration of amphibian tissues are conserved in humans, though their activity may be either suppressed or neutralized by inhibitory factors in humans or enhanced in amphibians. Thus, based on the knowledge that genes and their functions are highly conserved between species, using axolotls, with the aim of uncovering the mechanisms leading to perfect tissue regeneration, is justified. Interestingly, salamanders are tight-skinned like humans and are long-lived (> 10 years in captivity), which provides the possibility of following the impact of a treatment on the health of the animal over many years, if desired. These animals have been used extensively for epimorphic regeneration studies, as they represent the only vertebrates capable of regenerating highly complex structures (*e.g.*, a complete limb, including the bones, nerves, muscle, and skin) after amputation. Other vertebrates such as the zebrafish are also used for regeneration studies, as they can regenerate the extremities of their fin rays quite well, and they are amenable to genetic screening (zebrafish are also used extensively for developmental genetics studies because of the ability to perform genetic screens).²⁴ However, teleost fish, such as the zebrafish, are not capable of regenerating their fins if amputated proximal to the basal articulations of the rays,²⁵ and no report has ever been published on their ability to heal skin wounds as adults. Axolotls are not highly amenable for genetic screening, but there are more and more tools now available to perform research of a mechanistic nature in these animals. Transgenic axolotls have been generated with constitutive²⁶ and inducible promoters,²⁷ and specific microarrays are available for assessing the expression of thousands of genes.²⁸ In addition, since axolotls are normally aquatic salamanders,

Figure 2. Mature wild-type axolotl ~5 years of age (25-cm total length). External gills (#1) can be noticed, as this species of salamander remains aquatic for its entire life. The different numbers depict structures that can regenerate after injury or amputation throughout the animal's life: 1, gills; 2, parts of the brain; 3, jaws; 4, apex of the heart; 5, limbs, both fore- and hindlimbs, can regenerate when amputated distally or proximally; 6, the skin and some internal organs such as parts of the intestine and pancreas; 7, the tail and the spinal cord, whether transected, crushed, or even if a segment is removed. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

one can use the ever growing number of commercially available signaling pathway agonists and antagonists simply by adding them to the water to test the importance or role of various proteins on development and regeneration.^{29,30}

As the theme of the current issue of the journal is centered on wound healing and transforming growth factor beta (TGF- β) action, the remainder of the present review will focus on wound healing and skin regeneration in the axolotls with an emphasis on what is known about the role of TGF- β in this organism. For those interested more about limb regeneration, we suggest review articles by Brockes (1997),³¹ Roy and Lévesque (2006),²² and Tanaka and Reddien (2011).³² As mentioned previously, humans are tight-skinned animals, and finding a model organism that is also tight skinned presents some advantages when considering mechanisms of cell migration during wound healing and wound closure. We were the first group to publish an article that examined how skin wound healing in axolotl proceeds.³³ This study confirmed that axolotls do indeed heal full-thickness excisional skin wounds in a scarless manner. Re-epithelialization was very rapid with keratinocytes covering a 1.5 mm-diameter wound in 8 h, although this layer was not attached to the underlying muscles.³³ Incisional wounds were assessed, but since axolotls are so efficient at healing and regeneration, it was almost impossible to survey the healing process for such a type of wound.³³ A timecourse study of 90 days was performed to assess whether skin regeneration in axolotls was perfect with all the components of the skin, including the basal lamina, reconstituted to their

original state. The epidermal layer of the skin was returned to normal in <45 days, except for the basal lamina that was not fully reconstituted yet.³³ By 90 days postwounding, it was not possible to distinguish between injured and intact skin.

TGF- β 1 has been implicated in wound healing in mammals,³⁴ and we also demonstrated that it was expressed during the initial phases of limb regeneration in the axolotl at the time when epidermal cells migrate to cover the stump wound in the first 24 h.²⁹ In a previous review article, we highlighted the differences and similarities between the different phases of limb regeneration (limb regeneration can be divided in two main phases: a preparation phase and a redevelopment phase) and limb development.²² We also took our analysis a step further and compared it to the initial phases of wound healing and noted that many events observed during the early phase of limb regeneration after amputation, such as the formation of a blood clot, wound epithelium formation, and extracellular matrix remodeling, are similar to events occurring in skin wound healing in mammals.²² Normal wound healing in mammals is marked by a rapid recruitment of neutrophils at the wound site.³⁵ Axolotls and newts, however, display a somewhat reduced immune response and almost no inflammation after amputation.³⁶ This is similar to the immune response observed in mammalian fetal wound healing, which also heals perfectly.^{20,21} This prompted us to look at the presence of neutrophils at various time points after wounding. There were almost no neutrophils or very few, considering the size of the wound.³³ This was in line with Ziegels' observations that very few macrophages were present at 6 h postwounding.³⁷ Macrophages are normally recruited to the wound site after neutrophils. Interestingly, we observed that in the axolotl, epidermal cells start to migrate over the open wound as early as 1 h postwounding with almost no neutrophils in the area,³³ which contrasts with observations in mammals where neutrophils are recruited to the wound site immediately after injury and are present for a period of time before epidermal cells start to migrate over the wound. These experiments raised the question whether axolotls are capable of forming scar tissue at all. To answer this question, we took advantage of the fibrogenic potential of bleomycin and injected it subcutaneously to induce fibrosis in axolotls. We were able to demonstrate that indeed axolotls can produce scar tissue, although this scar tissue was devoid of collagen and consisted mostly of fibronectin.³³ To obtain a better understanding of the initial molecular events that unfold during

skin wound healing in axolotls versus mammalian models, the expression of four genes known to be expressed early in wound healing was determined. *TGF- β 1*, *Krox-24*, *Msx-1*, and *Hsp-70* were selected because they had been shown to be activated early in normal wound healing in mammals.^{38–41}

Figure 3 shows the expression of the aforementioned genes at early time points postwounding in the axolotl skin. The pattern of gene expression observed in the axolotl skin was similar to that reported in the mammalian skin, with a somewhat faster time course. The expression of *TGF- β 1* was in the wound itself, as expected, and peaked around 6 h postwounding. The expression of *Krox-24* started around the edge of the wound very early (as early as 2 h postwounding), again very similar to what has been reported in mice wounds, and then its expression became restricted to the wound site by 6 h postwounding, and finally was almost undetectable by 24 h. *Msx-1* had a similar pattern of expression to *TGF- β 1* with a peak at 6 h in the wound site and still some expression by 24 h. Finally, *Hsp-70* expression was similar to *Krox-24* at the 2-h time point and then was more like *TGF- β 1* and *Msx-1* by 24 h. One of the roles of *TGF- β 1* in scarring is associated with its ability to induce the expression of alpha-smooth muscle actin (α -SMA) in fibroblasts, facilitating their differentiation into myofibroblasts.⁴² Myofibroblasts are thought to be the cells responsible for the production of collagen bundles in mammalian wounds, leading to scarring. Since *TGF- β 1* was expressed in axolotl wound healing, we also examined the expression of α -SMA at various time points in excisional wounds in the axolotl, but found it to be absent.³³ This was later confirmed by the group of Maden at the University of Florida in Gainesville, who also studied the ability of axolotls to heal their wounds and found no evidence of α -SMA expression.⁴³ They also determined that collagen type III was expressed early during wound healing, and that collagen type I appeared later at the end of the healing process,⁴³ which is similar to the collagen expression pattern observed during fetal wound healing.²¹ As mentioned earlier, we demonstrated in a previous study that TGF- β signaling is essential for limb regeneration to proceed normally in axolotls.²⁹ We also demonstrated in that study that the TGF- β signaling machinery (*e.g.*, receptors type I and type II) was functional, and that axolotl cells responded to *TGF- β 1* by upregulating the expression of Smad3-responsive genes (*e.g.*, plasminogen activator inhibitor-I).²⁹ In fact, multiple studies have shown that TGF- β signaling is required for tissue regeneration in various animal

Figure 3. Whole-mount *in situ* hybridization on axolotl full-thickness excisional skin wounds was performed to look at the expression of four different genes known to be expressed early in mammalian wounds. An early time course for the expression of *TGF-β1*, *Krox-24*, *Msx-1*, and *Hsp-70* shows the expression of the messenger RNA (view is from the above the wounds). Dotted circles indicate the area of skin that was removed for the wounds with the dorsal side on top for each panel. The expression of each gene is shown at 30 min, 2, 6, and 24 h postwounding (the expression appears in dark around the circle). The wounds were done on the side of the tail at the midlevel with a 1.5-mm punch to excise a full-thickness piece of skin. Technical details for whole-mount *in situ* hybridization can be found in Lévesque *et al.* (2007).²⁹ To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

models: *Xenopus laevis*, zebrafish, and even invertebrate *Antedon mediterranea*.^{44–46} The other *TGF-β* isoforms were either not regulated (*TGF-β2* [Roy S, unpublished data]) or not detectable (*TGF-β3* was undetectable by polymerase chain reaction amplification or western blotting with various antibodies against this isoform [Roy S, unpublished data]) in axolotl regenerating tissues.

The fact that axolotls are normally neotenic (*i.e.*, they remain aquatic and do not metamorphose into a land-dwelling salamander unless exposed to thyroxine) has often raised the question of whether or not their ability to regenerate was due to their unique nature. The ability of axolotls to regenerate limbs independently of their metamorphic state has been demonstrated over three decades ago.⁴⁷ These experiments also demonstrate that regeneration is not dependent on an aquatic environment, as once metamorphosis is complete, axolotls are terrestrial, and they still regenerate.^{47,48} More recently, Maden's group, in their skin wound-

healing study, specifically addressed this point by inducing metamorphosis of adult axolotls and assessing skin regeneration/scar-free healing in pedomorphic versus metamorphic animals.⁴³ Their findings clearly demonstrate that metamorphosis does not affect wound healing in axolotls, and therefore supports the argument that axolotl regeneration is not a neotenic-dependent process unlike limb and tail regeneration in anurans (*i.e.*, frogs), which occurs only before metamorphosis.⁴⁹ Some anurans, however, can still regenerate their skin after metamorphosis, which may indicate that limb regeneration is somehow affected more by metamorphosis than scarless wound healing.⁵⁰ Finally, Maden's group also showed that the immune response after full-thickness skin excision is not changed significantly in pedomorphic versus metamorphic animals and remains relatively modest compared to what is observed in mammals.

There is no doubt the axolotl itself, as an animal model, is interesting because of its extraordinary

Figure 4. (a) Lower jaw of a 62-year-old male patient at the day of oral surgery. Silk sutures (black) were placed to close the large incisional wound created to the oral and gingival tissues for the removal of four teeth, insertion of two dental implants, and placement of bone grafts. The same surgical area is shown at (b) 2 weeks and (c) 4 months postoperatively. No scar tissue was clinically observed. (d) Insertion of a free keratinoconjunctival graft tissue (to augment the lack of keratinized tissue at future implant site). The same surgical area is shown at (e) 2 weeks and (f) 3 months postoperatively. The amount of postoperative scarring is low, considering the extent of the surgical procedure compared to a similar type of skin-grafting surgery. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

regenerative capacities. In addition, a recent study by Menger and colleagues suggest that this regenerative capacity may partly be due to the axolotl genes themselves. They examined whether the epidermal lipoxxygenase, a gene important for wound healing in mammals, of axolotl could influence wound closure in mammalian cells.⁵¹ They compared the ability of axolotl versus human lipoxxygenase to influence wound closure using scratch assays with different mammalian cell lines. They found that the axolotl lipoxxygenase was much more efficient than human lipoxxygenase in promoting wound closure in these *in vitro* assays.⁵¹ This finding may not be generalized for all axolotl genes, as we have shown in a previous study that the axolotl p53 protein had no activity at 37°C in human cells.³⁰ However, it remains interesting that some axolotl genes known to be important for normal wound healing are more efficient in their actions than their mammalian counterparts. Understanding what makes these axolotl genes perform better could provide insights for developing drugs or therapies to improve wound healing.

Scarless wound healing and/or skin regeneration in axolotl shares many striking points of resemblance with the only adult mammalian tissue capable of nearly perfect scarless wound healing: the oral mucosa. The oral mucosa, like limb regeneration and scarless wound repair in axolotls, displays a limited immune response with few neutrophils invading the wound site.⁵² It is interesting that wound healing occurs with a mild inflammatory response in an environment rich in bacteria such as the oral cavity. Again, TGF- β signaling is important during mammalian oral wound healing⁵³ as in limb regeneration in axolotls.²⁹ Although human oral wounds do not completely regenerate or heal perfectly, their level

and quality of healing observed after extensive surgery are quite amazing, considering the tissue damage resulting from these surgeries (see Fig. 4).

CONCLUSION

The axolotl, with its regenerative capacity, offers a unique model to analyze the potential interactions between signaling pathways modulating the regenerative process in vertebrates. The complexity of the regenerative process (wound healing, dedifferentiation, proliferation, extracellular matrix remodeling, and pattern formation) is such that it likely results from the convergence of multiple pathways leading toward tissue replacement. In addition, an attenuated immune response seems to correlate with scarless wound healing in mammals and amphibians^{20,21,36} and could be essential for regeneration. A better understanding of the process and hierarchal cascade of gene expression controlling cellular activity after amputation or injury in axolotls will yield important knowledge that may lead to the identification of genes or proteins that represent good targets for therapeutic intervention to improve tissue repair in mammals.

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TAKE-HOME MESSAGES

Basic science

- Various animal models are needed to help us understand the difference between scarring and regeneration.
- Each model has advantages and disadvantages (tight and loose skin; genetic manipulations or lack thereof).
- Evolutionary conservation of gene function allows us to study animals from divergent phylogenetic ancestry and transfer what is learnt from one group to another.
- TGF- β signaling is involved in both scarring and regeneration, which highlights the complexity of biological systems and their signaling pathways.
- The axolotl, even though it is a urodele amphibian, offers many advantages to identify the signals involved in tissue regeneration.

Relevance to clinical science

- Oral wound healing in humans is in many ways similar to regeneration in axolotls: low inflammation and minimal scarring.
- Identification of genes or pathways responsible for regeneration in vertebrates such as axolotls will provide targets for the development of therapeutic applications.

Unfortunately, due to the limited scope of this review article and space limitations, we were not able to refer to all the work that has been done on this subject. This work was supported by grants from the Canadian Institute of Health Research, the National Science and Engineering Research Council of Canada, and the Network for Oral and Bone Health research from the Fonds de Recherche du Québec-Santé to S.D.T. and S.R.

AUTHOR DISCLOSURE AND GHOSTWRITING

All the authors have participated in the writing of this article and declare no conflicts of in-

terest. There was no ghostwriting involved in this article.

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