Review

For reprint orders, please contact: reprints@futuremedicine.com

Regenerative Medicine

The strategy and method in modulating finger regeneration

Yohan Choi[1,2], Charles Cox[1], Kevin Lally[1] & Yong Li*[,1,2]

[1]Children's Regenerative Medicine, Department of Pediatric Surgery, University of Texas Medical School at Houston, TX 77030, USA

[2]Center for Stem Cell & Regenerative Medicine, University of Texas Health Science Center at Houston (UT Health), TX 77030, USA

*Author for correspondence:
Tel.: +1 713 500 2438
Fax: +1 713 500 2424
yong.li.1@uth.tmc.edu

The tip of the human finger can regenerate if the amputation is distal to the nail bed, usually in young children. Studies in regeneration of rodent digits have shown that regeneration occurs if the amputation is distal to the mid-third phalanx for certain ages. The digit contains many different components, such as muscle, tendon, bone, skin, nerves and blood vessels, which must all be regrown in the proper location in order to restore functionality. The mechanism behind the complex healing/regeneration processes is still under investigation; however, improvements in injured finger regeneration have been gradually developing in animal models over the past few years. This review discusses a few strategies and methods to possibly enhance digit regeneration beyond current natural limits, focusing on aspects including scarless wound healing, cell-based treatments, tissue engineering and electrical stimulation.

Keywords: electrical stimulation • finger regrowth • scarless wound healing • stem cell • tissue engineering

Finger injuries are very common. Hand and finger injuries account for one-third of work-related injuries [1]. In the USA, between 1990 and 2002, there were estimated to be 111,600 children younger than 18 years of age with amputation injuries, with finger amputations accounting for greater than 90% of all amputations [2]. Currently the best course of action for a digit amputation injury is replantation; however, whether the digit can be replanted or not will depend on microscopic inspection of vessels and nerves [3,4]. An alternative option is transplanting the patient's toe. If the digit cannot be replanted it would be ideal if it could be regenerated. Although it is possible to regenerate the very tips of amputated digits with fingerprint and nail restoration, and bone regrowth under certain conditions, digit regeneration is not currently possible for amputations proximal to the third phalanx [5–9]; however, it is conceivable that treatment with the right growth factors, progenitor cells, scarring inhibitors or some combination thereof would allow the latent developmental process to completely regrow an amputated digit or possibly even an entire appendage.

Perfect regeneration of limbs occurs in urodele amphibians, such as newts and salamanders. There are two major theories of why regeneration does not occur in mammals. One theory is that the regenerative program is suppressed as the immune system matures; in adult mammals, clearing pathogens appears to be evolutionarily favored compared with retaining the ability to regenerate [10]. The second theory is that there are genetic differences that either allow regeneration in salamanders and newts or prevent regeneration in mammals. Urodele amphibians may have retained regeneration-specific genes not found in mammals, which allow their cells to dedifferentiate [11]. Inhibition of regeneration in mammals could be due to tumor suppression genes, such as those found in the *Ink4a* genetic locus present in mammals but not amphibians, which encodes the tumor suppression genes *p16ink4a* and *ARF* [12]. Inactivation of both tumor suppressors Rb and ARF allowed terminally differenti-

Future Medicine part of fsg

ated mammalian muscle cells to dedifferentiate and re-enter the cell cycle [12]. Reactivation of the genes returned the cells to their differentiated state.

The first step after amputation injury in both regenerating and nonregenerating organisms is wound healing, which is a complicated process involving the interaction between many cell types, mediators and the surrounding environment. Wound healing can be divided into four major subcategories: hemostasis, inflammation, proliferation and remodeling (Figure 1) [13,14]. Mammalian wound healing often ends in fibrosis and scar formation. In regenerating injuries, the wound epidermis converts into a special layer of signaling cells called the apical epithelial cap (AEC) [15]. Skin, muscle, bone and other tissue local to the amputation site partially dedifferentiate into a blastema of proliferating cells, which serves as the cellular source for the newly regenerating limb [15]. After the blastema creates a sufficient mass of progenitor cells, redevelopment occurs through signals between the AEC and blastema. Dedifferentiation is thought to only occur in regenerating species; however, our laboratory has shown evidence of dedifferentiation after muscle injury in mice, indicating the potential for this event to occur in mammals [16]. The following sections will discuss the potential methods of accelerating finger regrowth with new techniques.

Stem cells & cell-based therapy

The anticipated outcome of stem cell therapy is the replacement of lost endogenous cells, integration with the host tissue and restoration or enhancement of lost function. Various cells have been used for therapeutic purposes to attempt treatment of injuries and disorders such as cardiovascular disease, spinal cord injury, diabetes, lung disease and numerous other ailments. There are three main types of stem cells that are being utilized for cell therapy: embryonic stem (ES) cells, adult stem cells and induced pluripotent stem (iPS) cells. Due to ethical concerns and the risk of uncontrolled growth, the enthusiasm for using ES cells for cell therapy has been reduced; nevertheless, clinical trials are underway using cells from existing human ES cell lines, such as those differentiated into retinal pigment epithelium to treat macular degeneration [17]. iPS cells can be created from somatic cells by ectopic expression of several transcription factors [18]. Although iPS cells can be converted into many useful cell types, two difficulties are tumor formation [19] and low efficiency of transformation while using nongenetic methods [20].

Most adult tissues contain resident stem cells to maintain homeostasis; however, many of these cells have limited proliferation and differentiation potential. Nevertheless, utilizing the patient's own adult stem cells has the advantage of decreasing the likelihood of immune rejection and having no ethical issues. Multipotent mesenchymal stromal cells (MSCs), which proliferate to form a heterogeneous population of fibroblast-like cells, can be obtained from many adult tissue sources; however, bone marrow, adipose tissue and umbilical cord are the most common sources. MSCs can be differentiated into many types of cells, such as adipocytes, osteocytes and chondrocytes *in vitro* [21]. Over 100 clinical trials using MSCs to treat a variety of disorders are in Phase I

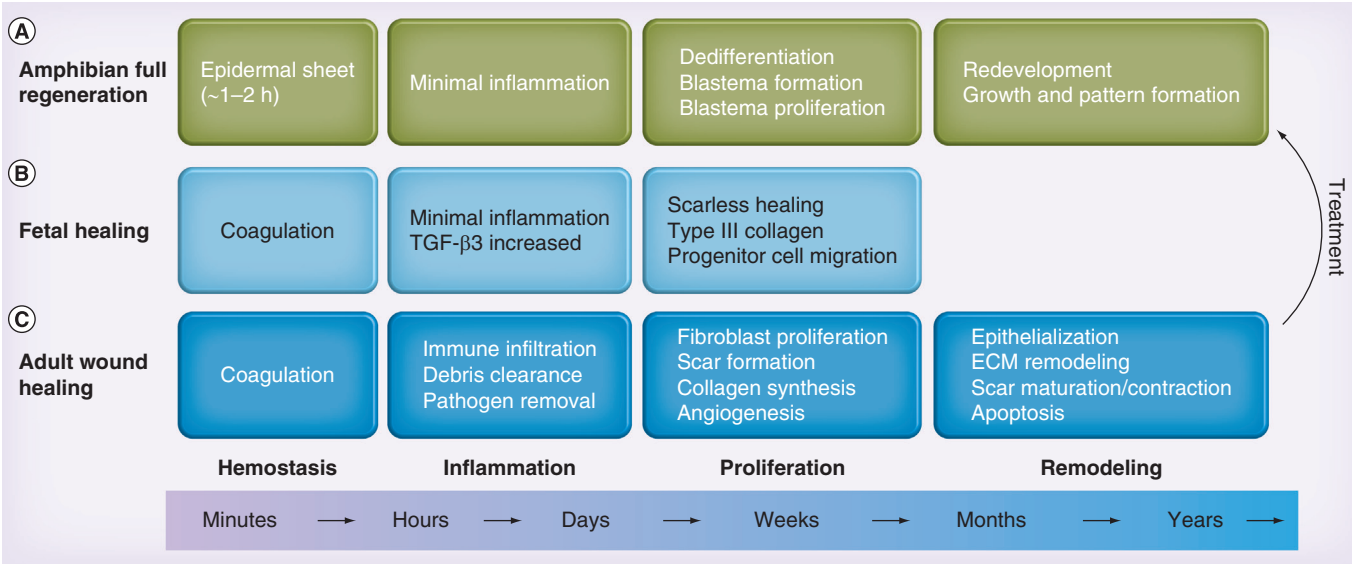

Figure 1. Stages of wound healing and regeneration (logarithmic time scale). The major phases of wound healing are hemostasis, inflammation, proliferation and remodeling. **(A)** Full regeneration in amphibians regrows damaged tissue. **(B)** Fetal healing of small wounds results in scarless healing. **(C)** Normal adult wound healing results in scar formation.
ECM: Extracellular matrix.

or II studies, with early results mixed, which may be due to extracellular factors [22]. In many cases, the implanted stem cells disappear (even if there was an observed therapeutic effect), suggesting that the stem cells play a role in activating a secondary response rather than being the effectors of the response [23,24]. In a wounding model, human MSCs recruited endogenous tissue stem cells from the host to enhance healing [24].

In a regenerating amphibian limb, the blastema is composed of heterogeneous cells that are less differentiated than the surrounding tissue. Regeneration in the mammalian digit tip may also form a blastema; however, there is some controversy over whether this is a true blastema or not [25,26]. One reason why regeneration of the digit is limited to the distal tip could be due to insufficient adult stem cells, some of which reside under the nail bed. Adult stem cells from underneath the nail bed may be a possible source for regenerating a digit tip [27]. Whether adding additional stem cells will enhance regeneration remains to be determined. The type or types of cells that would be optimum to enhance blastema growth is not known; however, care must be taken, as some cell types display uncontrolled tumor-like growth. One group reported transplantation of embryonic day 11–13 limb buds, the dissociated cells of limb buds or bone marrow-derived mesenchymal cells from a GFP mouse into neonatal mice that were amputated at the autopod level, with metacarpal bones removed [8]. Both the limb buds and dissociated cells showed development of digit-like structures, with more complex patterns shown with the later-staged limb buds. The dissociated cells were able to form the beginnings of a digit-like structure, indicating that some of the patterning information was still present in the cells; however, there appeared to be missing signals since the regeneration was far from complete. The bone marrow mesenchymal cells formed a cartilage pattern at the stump, suggesting that an alternative source of cells may enhance regeneration; however, no similar study has been reported. Whether utilizing other cells or a mixture of cell types enhances regeneration might be a topic for further investigation.

Scarless healing

The effect of scars on wound healing is clearly demonstrated by comparing fetal and adult wounds. Adult wounds normally heal with scar formation; fibrotic scar tissue is primarily comprised of type I and III collagen (reviewed in Mu *et al.* [28]). Adult wounds are rich in collagen I, which provides the strength and rigidity needed to keep the wound closed; however, it impedes migration of cells, leading to scar formation rather than regeneration. Fetal wounds, on the other hand, are rich in collagen III, which allows cellular migration through the matrix. Decreasing fibrotic scar tissue formation could presumably enhance wound healing as scar tissue only restores approximately 70% of the strength of the original tissue [29]. Scarless fetal healing displays differences in extracellular matrix (ECM) composition, inflammatory response, cellular mediators and gene expression profiles from postnatal, scarring wounds (reviewed by Larson *et al.* [10]). Although scarless healing is generally thought to be preferred to healing with scar tissue formation, in certain cases, the formation of an initial transient scar is beneficial for initial wound healing. For example, in the zebrafish cryoinjured heart, prevention of the initial scar reduced regeneration processes [30]. In an organ exposed to high amounts of force, the initial scar provides necessary support needed to maintain structural integrity.

Scarless healing was serendipitously found in the Murphy Roths Large (MRL) mouse, which is used as a model to study systemic lupis erythematosis [31]. The MRL mouse completely heals through-and-through ear punches; the ear hole closes and skin, cartilage and hair regrow. These MRL mice display much higher MMP-9 activity and slightly higher MMP-2 activity in healing ear tissue compared with nonhealing B6 mice due to lower TIMP expression in the MRL mice [32]. A p21-deficient mouse, which also displays a lupus-like syndrome, demonstrated the ability to completely heal through-and-through ear wounds. The MRL mouse similarly does not express the p21 protein, suggesting a link between cell cycle checkpoint control and tissue regeneration [33]. The neutrophils of MRL mice express higher amounts of MMP-2, -3 and -9 compared with C57BL/6 mice [34]. MMP-3 specifically degrades collagen type IV found in the basement membrane, which may help explain how the MRL mice remove the basement membrane and heal ear punches without scarring. Although the MRL mouse heals ear wounds without scarring, dorsal cutaneous wounds were found to form scars similar to other mouse strains [35,36]. A blastema-like structure was found in the regenerating ear wound of the MRL mouse but not in dorsal skin wounds, suggesting that location of injury plays a role in determination of repair or regeneration [37]. Why dorsal cutaneous wounds form scars while ear punches do not is unclear; however, the authors speculate that differences in architecture between the ear and back, as well as mechanical stress, may play a role in the differences in healing [36].

Environmental influences during fibrosis formation

In experiments where digits from 4-week old mice were amputated distal to the midpoint of the third phalanx, MRL mice demonstrated quicker regrowth compared

with C57B inbred mice controls [38]; however, when the digits from adult mice (6–8 weeks or 3–4 months) were amputated at the midpoint of the second phalanx, neither MRL mice nor controls regenerated their digit tips [39,40]. Interestingly, only the MRL mice displayed a blastema-like formation during the early stage after amputation with numerous proliferating cells; however, an apoptotic event causes these cells to disappear [40]. Understanding how the blastema-like structure forms and preventing the apoptotic event from occurring might allow the cells in the blastema to proliferate enough to induce regeneration in an amputation of the second phalanx. The presence of nerve axons are required for formation of the blastema and regeneration to occur [41]. However, addition of FGF2 to a denervated digit restored regenerative capacity, suggesting that nerve axons stimulate FGF2-dependent blastema formation [27]. FGF signaling may play a role in blastema growth, since exogenous addition of FGF2 to cells collected from a digit blastema greatly increased proliferation [40]. Thus, addition of FGF2 to a mid-second phalanx-amputated digit may result in partial regeneration.

TGF-β is a growth factor associated with scar formation via proliferation and migration of fibroblasts, collagen deposition and matrix metalloproteinase (MMP) inhibition. Although high levels of TGF-β1 are associated with inflammation, treatment of proliferation-arrested myoblasts with a low level (0.5 ng/ml) of TGF-β1 increased stem cell-like properties [42]. TGF-β3 expression is increased, while TGF-β1 expression is unchanged in scarless fetal wounds compared with adult scar forming wounds. Conversely, fetal wounds that do form scars show increased TGF-β1 and -β2 expression, with decreased TGF-β3 expression [43]. A *TGFBR1* mutant showed increased regenerative capacity, as demonstrated by complete closure of a through-and-through hole punch in the ear [44]. It was hypothesized that the point mutation caused partial activation of the receptor, since there was an increase of some TGF-β target genes. These *TGFBR1* mice also showed faster differentiation of bone marrow stromal cells into chondrogenic precursors. Mice deficient in Smad3, a downstream mediator of TGF-β, demonstrated accelerated wound healing and reduced local inflammation in incisional dorsal skin wounds [45]. However, wounds in the ear did not demonstrate changes in inflammatory cells, and the ear wounds enlarged rather than decreased in size [46].

Inflammatory cells are numerous in adult wound healing, while few in number in scarless fetal wound healing [10]. Scar-free healing was demonstrated in the adult *PU.1* null mice, which lacks many inflammatory cells (macrophages, neutrophils and T and B cells) [47]. Cluster analysis of genes expressed after wounding in *PU.1* null mice compared with wild-type siblings highlighted differences between tissue repair genes and those associated with the inflammation process that are not directly necessary for wound healing [48]. However, depleting inflammatory cells does not always lead to better healing. After acute muscle injury, the presence of inflammatory macrophages leads to IGF-1 production and improved muscle repair compared with muscles lacking macrophages [49]. Macrophages, as well as other inflammatory cells, release various cytokines. Proinflammatory cytokines IL-6 and -8 are upregulated in scarring wounds, while scarless wounds express the anti-inflammatory cytokine IL-10. Viral overexpression of IL-10 induced scarless wound healing in adult mouse wounds, although in both reports, expression of IL-10 was induced before wounding [50,51]. Conversely, IL-10-deficient mice demonstrated scar formation when injured in the normally scarless fetal stage [52].

Scarless skin wound healing in FOXN1-deficient mice was correlated with increased MMP-9 and -13 expression, along with rapid collagen turnover [53]. Both MMPs were found to have a bimodal expression in mutant mice, with increased expression in the epidermis during the early (inflammatory) phase and again during the late (remodeling) phase. Control mice, which healed with scarring, displayed only the initial peak in MMP-9 and -13 (Figure 2). The authors speculated that release of MMP-9 and -13 by mutant fibroblasts in the late healing phase assists in migration of these fibroblasts, along with collagen remodeling, which, in turn, leads to scarless healing. Previous studies by our laboratory indicated that MMP-1 treatment both decreased scar formation and improved myofiber regeneration during healing from laceration injury of murine muscle [54]; therefore, we hypothesized that MMP-1 treatment of amputated digits would augment regeneration. MMP-1 was added after digit amputation in the mid-second phalanx to ascertain if digit regeneration would be enhanced [55]. Treatment of amputated digits with MMP-1 resulted in regrowth of soft tissue, with decreased formation of fibrotic tissue. Although the digit did not regenerate with MMP-1 treatment, the speed of wound closure, capillary vasculature formation and peripheral nerve fibers and neuromuscular junction formation all improved compared with nontreated digits. In addition, MMP-1 treatment increased the number of Sca-1-positive progenitor cells.

Treatment of fingertip or other injuries with scar-reducing compounds may enhance healing and regeneration. A list of factors affecting scarless healing is given in Table 1. If a soluble compound could be injected or applied to the amputation site to reduce

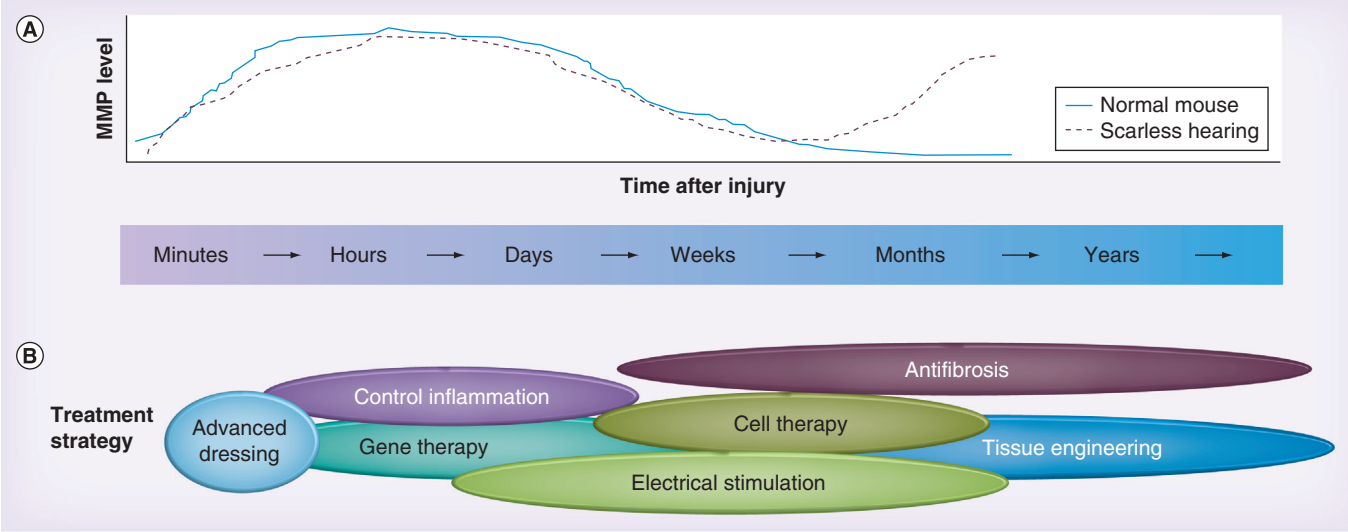

Figure 2. Regenerative response. (A) Growth factor levels after injury. After injury, MMP levels in a normal mouse (solid line) decreases after an initial peak; however, scarless *Foxn1* null mice (dotted line) have a second burst of MMP expression. **(B)** Possible treatment strategy to convert from scar formation to regeneration.
MMP: Matrix metalloproteinase.
(A) Data taken from [53].

scarring, regeneration might be enhanced. Relaxin is a circulating peptide involved in remodeling of ligaments during gestation, which was found to promote muscle healing and reverse scar formation [56,57]. The mechanism of action is still under investigation; however, one means of regulating the ECM appears to be via activation of MMPs. The use of other antifibrotic agents such as D-penicillamine, colchicine, immunomodulatory agents and biological drugs to reduce scarring is a topic of ongoing research [58].

ECM treatment & tissue engineering

The ECM has been used as a biological scaffold material for numerous tissue engineering applications, such as for the bladder and esophagus [59]. ECM powder from porcine intestines (MatriStem®; ACell, MD, USA) was reported in various news stories to regrow the amputated fingertips of adults [60–62]. However, whether or not the fingertips would have regrown naturally without ECM treatment and if the distal phalangeal bone regrew are unclear. Treatment of murine digits amputated in the middle of the second phalanx with ECM powder demonstrated recruitment of endogenous stem cells to the injury site; however, regeneration did not occur [63]. The cells isolated from ECM treatment were able to be induced into neuroectodermal and mesodermal lineages *in vitro*, while cells from control digits were only inducible into mesodermal linages, suggesting that ECM degradation products recruited multipotent stem cells to the injury site or facilitated dedifferentiation. A follow-up study by the same group demonstrated similar results when mid-second phalanx-amputated digits were treated with a single peptide derived from the α-subunit of collagen, with increased expression of the stem cell markers Sox2 and Sca1 found in cells isolated from treated digits [64]. As MMPs degrade collagens, this could represent a possible avenue for how MMP enhances digit regeneration.

Decellularized tissue has been used as a scaffold to grow organs, such as a beating heart [65]. The cells were removed by detergent, leaving a 3D ECM scaffold that was seeded with progenitor cells. Whether a cadaveric digit or limb could also be decellularized, reseeded with cells from the patient and then transplanted remains an intriguing question. It is conceivable that the ECM could be attached to the stump in sections for regrowing an entire limb. Although the technology to attach the scaffold of an entire limb in a bioreactor and provide the nutrients and cellular support is not yet available, research is quickly progressing. Use of synthetic biomaterials as an ECM for tissue engineering is a rapidly growing field and has been reviewed by Lutolf and Hubbell [66]. How the ECM would be attached to the wound, the best type or types of matrix and the optimum selection of progenitor cells to seed the scaffold remain to be determined.

There have been several studies that have attempted to tissue engineer entire phalanges with stem cells and biodegradable scaffolds. Implantation into immunodeficient mice allowed the simulated transplantation of the tissue-engineered phalanges, with formation of bone, cartilage and blood vessels assayed. One group obtained stem cells from bovine periosteum, cartilage

Table 1. Scarless healing.

Study (year)	Factor	Effect	Mechanism	Ref.
Clark *et al.* (1998); Bedelbaeva *et al.* (2010)	MRL mouse and *P21* knockout	Scarless healing of ear scar on cutaneous back wound	Lower TIMP expression; cell cycle checkpoint control	[31,33]
Gawronska-Kozak (2011)	*Foxn1* knockout	Scarless skin wound healing	Increased MMP-9 and -13	[53]
Liu *et al.* (2011)	TGFBR1 mutant	Scarless healing of ear	Increase of TGF-β?	[44]
Arany *et al.* (2006)	*Smad3* knockout	Accelerated healing of dorsal wound; ear wounds increased in size	ECM and wound closure	[46]
Gordon *et al.* (2008); Peranteau *et al.* (2008)	Overexpression of IL-10	Scarless wound healing	Decreased inflammation	[50,51]
Martin *et al.* (2003); Cooper *et al.* (2005)	*PU.1* null mouse (without macrophages or neutrophils)	Scarless wound healing to small paw wounds and dorsal wounds	No inflammatory response	[47,48]
Mu *et al.* (2010)	Addition of relaxin to injured muscle	Decreased scar formation	MMP expression and satellite cell mobilization	[56]
Bedair *et al.* (2007); Mu *et al.* (2013)	Addition of MMP1 to injured muscle and amputated digit	Less scar tissue, increased myofiber regeneration	Collagen breakdown; stem cell activation	[54,55]

ECM: Extracellular matrix; MMP: Matrix metalloproteinase; MRL: Murphy Roths Large.

and tendons, which were seeded onto biodegradable polymer scaffolds molded to resemble a human phalange [67]. The cells plus scaffold were cultured *in vitro* before implantation into nude mice. Over periods of up to 60 weeks, the implants recruited host vasculature and formed new bone, cartilage, and/or tendon. Another laboratory used a casting method to form the scaffold composed of a mixture of polylactic acid and polyglycolic acid [68]. The scaffold was wrapped with bovine periosteum and chondrocytes were injected into one end. After 8 or 16 weeks, the implants showed evidence of bone and cartilage growth with vascularized areas. A third group utilized a 3D printing method to generate a scaffold composed of β-tricalcium phosphate/poly(lactic-co-glycolic acid) [69]. Ultra-high-resolution volumetric computed tomography images of a human thumb were used as the blueprint for the 3D-printed scaffold, allowing construction of customizable shapes according to the needs of the patient. A mixture of CD 117^{+}-enriched human bone marrow-derived mesenchynal stem cells suspended in hydrogel was added to the scaffold, which was then implanted into nude mice for 1, 2, 4 or 6 weeks. Growth of new bone-like ECM demonstrated the potential of utilizing tissue engineering to generate phalanges.

Electrical stimulation

Electrical stimulation is thought to accelerate or restart the wound healing process by mimicking the natural electric current that occurs within damaged epithelium. Polarized ion transport across tight junctions generates an electric potential difference across the epithelium. A wound causes a low-resistance pathway that short-circuits the epithelial battery, generating current flow near the injury location [70]. The treatment of chronic wounds with applied electric fields with low, physiological current levels have been investigated in numerous clinical trials; a meta-analysis of 15 studies using various types of devices (continuous direct current, pulsed direct current and transcutaneous electrical nerve stimulation) reported an average 144% increase in the rate of healing chronic wounds when receiving some type of electrical stimulation compared with controls [71]. Applied electrical fields increased functional recovery in spinal cord injuries in rats, dogs and other animals [72–74], which has led to a Phase I clinical trial [75]. Bone repair can also be enhanced via electrical stimulation, which regulates ECM synthesis, gene expression and growth factor production, and can mend fractures that are otherwise refractory to healing [76]. Electrical stimulation in the amputated rat foreleg at the level of the humerus showed limited cancellous bone regrowth compared with controls, which showed no bone regrowth [77]. Similarly, amputated rat limbs with implanted fetal nerve tissue implants and electrical stimulation also induced bone regrowth, but not full regeneration [78]. Whether amputated digits would benefit from similar treatment or if the technique could be modified to increase regeneration beyond a short length of bone regrowth remains to be determined. The method by which electrical stimulation leads to improved wound healing or bone repair is not completely understood; however, some factors that may lead to improved healing, which are induced by

electric fields, are cellular movement and migration, differentiation and cellular proliferation [79].

Polarity of the electric field is also important at both the cellular and tissue level. Quiescent, terminally differentiated cells are electrically polarized, while stem and tumor cells are generally depolarized [80]. Nonregenerating wounds display a positive polarity throughout the healing process, while in regenerating animals, the polarity is initially positive but then quickly changes to negative [79]. The injury current in regenerating limbs can last for weeks, signifying that the field is not due to passive ion leakage. By contrast, the current decreases slowly as the limb heals in nonregenerating injuries. *Xenopus* embryos exposed to an electric field such that the net current flux was reversed in the embryo from its natural field showed severe developmental abnormalities [81]. Normally regenerating salamander limbs no longer regenerate if the electric field is reduced by Na^+-blocking agents or by forcing the electric field to reverse via implanted electrodes [82]. Similarly, unamputated newt limbs were induced to dedifferentiate by application of an electric field strong enough to induce electroporation but not cause necrosis or apoptosis [83]. Whether cellular polarization is the cause or result of dedifferentiation remains to be investigated.

Bone regrowth

Complete healing of amputated digits requires bone regrowth. Fingertip injuries with documented bone regrowth have been reported; however, the regrowth was limited to the distal tip. A more severe injury would presumably require additional osteoinductive factors to induce bone regrowth. Developmental bone growth occurs by endochondral ossification, where cartilage divides and forms bone. However, it was shown that bone formation in regenerating digit tips occurs by direct ossification of blastema cells and does not involve chondrogenic cells [84].

BMP7 administered in a gelatin matrix successfully induced bone and cartilage formation in mice amputated at the autopod level (palm), which is much below the level of normal regeneration [8]. Amputation at the zeugopod level (wrist) with distal radius and ulna removed in neonatal mice demonstrated bone regrowth with administration of BMP-7 and to a lesser extent, BMP-2; however, BMP-3, -4 and -6 did not induce bone regrowth in this model [85]. Addition of a hedgehog agonist (Hh-Ag 1.8) promoted bone formation when coupled with BMP-7, with the bone growth pattern slightly altered. Whether bone would regrow with BMP addition if the amputation plane was at the same level as the bone removal was not tested in this study. However, a different group studied the effect of BMP on regeneration of the digit tip. BMP-7 or -2 administered in an agarose bead elicited bone regrowth after amputation in the proximal portion of the third phalanx (just beyond the region for normal tip regeneration), but administration of BMP-4 did not lead to bone regrowth [86]. Treatment with BMP-7 resulted in longer digits compared with BMP-2 treatment, almost matching the unamputated control. Whether BMP addition would induce regeneration in adult mice or in more proximal amputation is not yet known.

Dental pulp stem cells (DPCs) are multipotent stem cells that have the capacity to differentiate into multiple lineages [87]. Autologous DPCs in a collagen sponge scaffold were used for human bone tissue repair [88]. A 3-year follow-up revealed that DPCs regenerated a compact bone, suggesting the use of autologous DPCs to generate bone in structures requiring higher matrix density, such as a digit phalanx [89].

Other methods of inducing bone regrowth include gene therapy using viral or nonviral vectors, or utilizing progenitor cells derived from bone marrow, fat, muscle and skin induced by the osteogenic pathway via genetic modification or exposure to growth factors (review by Ishihara and Bertone [90]). Adenovirus-mediated expression of 14 different BMPs *in vivo* demonstrated that BMP-2, -6, -7 and -9 induced ectopic bone formation in nude mice, with BMP-6 and -9 showing the greatest orthotopic ossification [91]. BMPs expressed via adenoviral vectors in nude rats showed bone growth (ordered from most to least) with BMP-6, -4, -9, -2 and -7, while immunocompetent rats demonstrated bone growth only with BMP-9 and -6 [92]. Tissue engineering approaches to creating bone implants that will support bone growth was mentioned above and include the use of biodegradable polymers, such as poly(fumaroyl bioxirane) maleate [93] or biomimetic nanofibrous scaffolds (reviewed by Holzwarth and Ma [94]). Injection of BMP-2 in a hyaluronic acid hydrogel was shown to be a minimally invasive technique for producing *de novo* formation of bone tissue in rats [95]. BMP-2 quickly breaks down when injected directly into the body, so the hydrogel serves as a nonimmunogenic carrier. Although BMP-2 expressed by an adenovirus did not induce ectopic bone growth in Sprague Dawley rats, BMP-2 delivered within a hydrogel did; this demonstrates the importance of the delivery method of exogenous growth factors.

Conclusion

Advancing digit regeneration beyond the current natural limitation may allow insights into discovering methods of regenerating an entire digit or even limb, or a nonregenerating organ. Digit tip regeneration is probably possible due to stem cells from the nail bed; how-

ever, such a source is not available in more proximal digit amputation or whole-limb amputation. Whole-limb regeneration might require an additional source of stem cells as well as a method to make sure these stem cells grow into the correct structure. Methods of altering the wound environment to prevent scar formation and promote tissue regeneration along with augmenting healing via extra stem/progenitor cells may assist this process. With advances in knowledge in urodele amphibian limb regeneration as well as development of the mammalian limb, the ability to regenerate human limbs beyond the fingertip is moving from the realm of science fiction and mythology into a possible reality.

Future perspective

Many methods of treatment of fingertip injuries and amputation have been employed, ranging from simple and biological dressings to terminalization and complex surgical procedures, such as cross-finger flaps and replantation. Some of the challenges to human limb regeneration are wound healing without fibrotic scar tissue formation, encouraging the formation, dedifferentiation and redevelopment of the blastema into a complete, functional limb. Some of the methods that might lead from scarring to perfect regeneration in humans are: cell-based therapy, gene therapy, treatment with small molecules, biomimetic scaffolds, mechanical forces (such as negative pressure) and electrical manipulation (Figure 2B) [96]. Scarless healing might be obtained by modulating the immune response via antibodies or peptides that target inflammatory agents such as TGF-β1 and increasing anti-inflammatory factors such as IL-10. Also, reduction of fibrotic scar formation may be accomplished by administration of substances such as relaxin or MMP-1 [55,97,98]. Why the digit tip will only regenerate in areas distal to the middle of the terminal phalanx is still not completely clear. Regeneration may require a certain number of adult stem cells from the nail bed or other factors that facilitate regeneration. Viral or nonviral gene therapy to enhance proliferation or inhibit tumor suppressors might encourage cells close to the wound edge to dedifferentiate and proliferate, although nonviral methods such as electroporation or gene gun delivery might be better suited to directly targeting cells in the regenerating stump. BMP treatment of neonatal mice was shown to induce a digit tip amputation that would normally end in a stub to regenerate [86]; whether this regenerative effect would extend beyond the first joint remains to be determined. Other growth factors or combinations might be able to extend regeneration to include the entire finger, hand or limb. Although the mammalian blastema shares some similarities with its amphibian analog, there are considerable differences. Mammals form only a limited blastema; increasing the blastema by inducing dedifferentiation of cells, re-entry into the cell cycle and prevention of blastema apoptosis might assist regeneration by production of more progenitor cells. Components of salamander blastema extract, which is able to dedifferentiate mammalian myofibers [99], might be applied to the amputation site to increase the number of adult stem cells. Perhaps stem cells derived from the patient might enhance regeneration (e.g., bone marrow or muscle-derived stem cells may be expanded *ex vivo* and then reintroduced into the amputation site). Directing the cells of the blastema to regrow an entire finger, hand or limb may depend on delivering signals from a bioengineered AEC equivalent, which could also serve as a dressing to rapidly cover the wound. While axolotl wounds are covered very rapidly, within 2–12 h after amputation [100,101], mammalian wounds take days to cover. This process may be accelerated by application of an artificial 'skin' over the wound, an ECM sheet or a simple occlusive dressing. However, closing the wound with a skin graft will prevent regeneration [6]. A regenerative sleeve covering the wound stump that acts as a bioreactor to control the physiological state of wound cells has been proposed [79]. Such a device would utilize

Executive summary

Cell therapy

- Regeneration requires a source of progenitor cells to regrow an amputated digit or limb. Adult stem cells may be a possible source.

Scarless healing

- Scar formation prevents regeneration. Inhibiting scarring via small molecules or genetic manipulation could be possible.

Tissue engineering

- A decellularized or biodegradable extracellular matrix could provide a platform for cells to attach and grow.

Electrical stimulation

- Electrical stimulation may enhance extracellular matrix synthesis, gene expression and growth factor production to enhance healing/regeneration.

Conclusion

- Insights into regenerating a digit could feasibly lead to regeneration of an entire hand or limb.

pharmacological, optical, electrical and genetic means to trigger regeneration and control patterning; however, more detailed information about the bioelectric states that promote regeneration is needed. A recent paper by Gardiner and Holmes suggested that nubbins may be an incomplete form of mammalian regeneration in the fetus [102], implying that the genetic program of regeneration exists in mammals. This further suggests that if certain conditions were improved or altered, complete regeneration could be possible.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1 Marty J, Porcher B, Autissier R. [Hand injuries and occupational accidents. Statistics and prevention]. *Ann. Chir. Main* 2(4), 368–370 (1983).

2 Hostetler SG, Schwartz L, Shields BJ, Xiang H, Smith GA. Characteristics of pediatric traumatic amputations treated in hospital emergency departments: United States, 1990–2002. *Pediatrics* 116(5), e667–e674 (2005).

3 Sebastin SJ, Chung KC. A systematic review of the outcomes of replantation of distal digital amputation. *Plast. Reconstr. Surg.* 128(3), 723–737 (2011).

4 Morrison WA, McCombe D. Digital replantation. *Hand Clin.* 23(1), 1–12 (2007).

5 Douglas BS. Conservative management of guillotine amputation of the finger in children. *Aust. Paediatr. J.* 8(2), 86–89 (1972).

6 Illingworth CM. Trapped fingers and amputated finger tips in children. *J. Pediatr. Surg.* 9(6), 853–858 (1974).

7 Neufeld DA, Zhao W. Phalangeal regrowth in rodents: postamputational bone regrowth depends upon the level of amputation. *Prog. Clin. Biol. Res.* 383A, 243–252 (1993).

8 Masaki H, Ide H. Regeneration potency of mouse limbs. *Dev. Growth Differ.* 49(2), 89–98 (2007).

9 Singer M, Weckesser EC, Geraudie J, Maier CE, Singer J. Open finger tip healing and replacement after distal amputation in rhesus monkey with comparison to limb regeneration in lower vertebrates. *Anat. Embryol. (Berl.)* 177(1), 29–36 (1987).

10 Larson BJ, Longaker MT, Lorenz HP. Scarless fetal wound healing: a basic science review. *Plast. Reconstr. Surg.* 126(4), 1172–1180 (2010).

11 Stocum DL, Cameron JA. Looking proximally and distally: 100 years of limb regeneration and beyond. *Dev. Dyn.* 240(5), 943–968 (2011).

12 Pajcini KV, Corbel SY, Sage J, Pomerantz JH, Blau HM. Transient inactivation of Rb and ARF yields regenerative cells from postmitotic mammalian muscle. *Cell Stem Cell* 7(2), 198–213 (2010).

• **Suggested that differentiation of mammalian muscle cells can be reversed back to lineage progenitors by inactivation of *Arf* and *Rb* genes.**

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

13 Broughton G 2nd, Janis JE, Attinger CE. The basic science of wound healing. *Plast. Reconstr. Surg.* 117(Suppl. 7), S12–S34 (2006).

14 Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. Dynamic reciprocity in the wound microenvironment. *Wound Repair Regen.* 19(2), 134–148 (2011).

15 Bryant SV, Endo T, Gardiner DM. Vertebrate limb regeneration and the origin of limb stem cells. *Int. J. Dev. Biol.* 46(7), 887–896 (2002).

16 Mu X, Peng H, Pan H, Huard J, Li Y. Study of muscle cell dedifferentiation after skeletal muscle injury of mice with a Cre-Lox system. *PLoS ONE* 6(2), e16699 (2011).

• **Discovered dedifferentiation that is often seen in more basal life forms, such as worms and amphibians, can also be detected in mammals, indicating a dedifferentiation process involved in wound healing of the injured murine skeletal muscles.**

17 Schwartz SD, Hubschman JP, Heilwell G *et al.* Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet* 379(9817), 713–720 (2012).

18 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4), 663–676 (2006).

19 Knoepfler PS. Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. *Stem Cells* 27(5), 1050–1056 (2009).

20 Zhou H, Wu S, Joo JY *et al.* Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 4(5), 381–384 (2009).

21 Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat. Rev. Immunol.* 8(9), 726–736 (2008).

22 Trounson A, Thakar RG, Lomax G, Gibbons D. Clinical trials for stem cell therapies. *BMC Med.* 9, 52 (2011).

23 Bedi SS, Hetz R, Thomas C *et al.* Intravenous multipotent adult progenitor cell therapy attenuates activated microglial/macrophage response and improves spatial learning after traumatic brain injury. *Stem Cells Transl. Med.* 2(12), 953–960 (2013).

24 Shin L, Peterson DA. Human mesenchymal stem cell grafts enhance normal and impaired wound healing by recruiting existing endogenous tissue stem/progenitor cells. *Stem Cells Transl. Med.* 2(1), 33–42 (2013).

25 Neufeld DA. Partial blastema formation after amputation in adult mice. *J. Exp. Zool.* 212(1), 31–36 (1980).

26 Muneoka K, Allan CH, Yang X, Lee J, Han M. Mammalian regeneration and regenerative medicine. *Birth Defects Res. C Embryo Today* 84(4), 265–280 (2008).

27 Takeo M, Chou WC, Sun Q *et al.* Wnt activation in nail epithelium couples nail growth to digit regeneration. *Nature* 499(7457), 228–232 (2013).

•• **Indicated the essential role of nail stem cells (NSCs) that reside in the proximal nail matrix and demonstrated that the mechanisms governing NSC differentiation are coupled directly with their ability to orchestrate digit regeneration. They also discovered the Wnt-dependent differentiation of NSCs and the necessity of Wnt activation for nail and digit regeneration after amputation injury.**

28 Mu X, Bellayr I, Walters T, Li Y. Mediators leading to fibrosis – how to measure and control them in tissue engineering. *Oper. Tech. Orthop.* 20(2), 110–118 (2010).

29 Madden JW, Peacock EE Jr. Studies on the biology of collagen during wound healing. 3. Dynamic metabolism of scar collagen and remodeling of dermal wounds. *Ann. Surg.* 174(3), 511–520 (1971).

30 Chablais F, Jazwinska A. The regenerative capacity of the zebrafish heart is dependent on TGFβ signaling. *Development* 139(11), 1921–1930 (2012).

31 Clark LD, Clark RK, Heber-Katz E. A new murine model for mammalian wound repair and regeneration. *Clin. Immunol. Immunopathol.* 88(1), 35–45 (1998).

32 Gourevitch D, Clark L, Chen P, Seitz A, Samulewicz SJ, Heber-Katz E. Matrix metalloproteinase activity correlates with blastema formation in the regenerating MRL mouse ear hole model. *Dev. Dyn.* 226(2), 377–387 (2003).

•• **Demonstrated the disappearance of the extracellular matrix and basement membrane in the healing Murphy Roths Large ear wounds (a well-known wound healing model) before blastema formation and examined MMP-2 and-9 expression, activity and cellular location during ear hole closure. This finding supported the fact that enzyme activity and balance (MMP/TIMP) are essential to wound healing.**

33 Bedelbaeva K, Snyder A, Gourevitch D *et al.* Lack of p21 expression links cell cycle control and appendage regeneration in mice. *Proc. Natl Acad. Sci. USA* 107(13), 5845–5850 (2010).

34 Heber-Katz E, Gourevitch D. The relationship between inflammation and regeneration in the MRL mouse: potential relevance for putative human regenerative(scarless wound healing) capacities? *Ann. NY Acad. Sci.* 1172, 110–114 (2009).

35 Colwell AS, Krummel TM, Kong W, Longaker MT, Lorenz HP. Skin wounds in the MRL/MPJ mouse heal with scar. *Wound Repair Regen.* 14(1), 81–90 (2006).

36 Beare AH, Metcalfe AD, Ferguson MW. Location of injury influences the mechanisms of both regeneration and repair within the MRL/MpJ mouse. *J. Anat.* 209(4), 547–559 (2006).

37 Rajnoch C, Ferguson S, Metcalfe AD, Herrick SE, Willis HS, Ferguson MW. Regeneration of the ear after wounding in different mouse strains is dependent on the severity of wound trauma. *Dev. Dyn.* 226(2), 388–397 (2003).

38 Chadwick RB, Bu L, Yu H *et al.* Digit tip regrowth and differential gene expression in MRL/Mpj, DBA/2, and C57BL/6 mice. *Wound Repair Regen.* 15(2), 275–284 (2007).

39 Turner NJ, Johnson SA, Badylak SF. A histomorphologic study of the normal healing response following digit amputation in C57bl/6 and MRL/MpJ mice. *Arch. Histol. Cytol.* 73(2), 103–111 (2010).

40 Gourevitch DL, Clark L, Bedelbaeva K, Leferovich J, Heber-Katz E. Dynamic changes after murine digit amputation: the MRL mouse digit shows waves of tissue remodeling, growth, and apoptosis. *Wound Repair Regen.* 17(3), 447–455 (2009).

41 Stocum DL. The role of peripheral nerves in urodele limb regeneration. *Eur. J. Neurosci.* 34(6), 908–916 (2011).

42 Mu X, Li Y. Conditional TGF-beta1 treatment increases stem cell-like cell population in myoblasts. *J. Cell. Mol. Med.* 15(3), 679–690 (2011).

43 Hsu M, Peled ZM, Chin GS, Liu W, Longaker MT. Ontogeny of expression of transforming growth factor-beta 1 (TGF-beta 1), TGF-beta 3, and TGF-beta receptors I and II in fetal rat fibroblasts and skin. *Plast. Reconstr. Surg.* 107(7), 1787–1794; discussion 1795–1786 (2001).

44 Liu J, Johnson K, Li J *et al.* Regenerative phenotype in mice with a point mutation in transforming growth factor beta type I receptor (TGFBR1). *Proc. Natl Acad. Sci. USA* 108(35), 14560–14565 (2011).

45 Ashcroft GS, Yang X, Glick AB *et al.* Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat. Cell Biol.* 1(5), 260–266 (1999).

46 Arany PR, Flanders KC, Kobayashi T *et al.* Smad3 deficiency alters key structural elements of the extracellular matrix and mechanotransduction of wound closure. *Proc. Natl Acad. Sci. USA* 103(24), 9250–9255 (2006).

47 Martin P, D'Souza D, Martin J *et al.* Wound healing in the *PU.1* null mouse – tissue repair is not dependent on inflammatory cells. *Curr. Biol.* 13(13), 1122–1128 (2003).

48 Cooper L, Johnson C, Burslem F, Martin P. Wound healing and inflammation genes revealed by array analysis of 'macrophageless' *PU.1* null mice. *Genome Biol.* 6(1), R5 (2005).

49 Lu H, Huang D, Saederup N, Charo IF, Ransohoff RM, Zhou L. Macrophages recruited via CCR2 produce insulin-like growth factor-1 to repair acute skeletal muscle injury. *FASEB J.* 25(1), 358–369 (2011).

50 Gordon A, Kozin ED, Keswani SG *et al.* Permissive environment in postnatal wounds induced by adenoviral-mediated overexpression of the anti-inflammatory cytokine interleukin-10 prevents scar formation. *Wound Repair Regen.* 16(1), 70–79 (2008).

51 Peranteau WH, Zhang L, Muvarak N *et al.* IL-10 overexpression decreases inflammatory mediators and promotes regenerative healing in an adult model of scar formation. *J. Invest. Dermatol.* 128(7), 1852–1860 (2008).

52 Liechty KW, Kim HB, Adzick NS, Crombleholme TM. Fetal wound repair results in scar formation in interleukin-10-deficient mice in a syngeneic murine model of scarless fetal

wound repair. *J. Pediatr. Surg.* 35(6), 866–872; discussion 872–863 (2000).

53 Gawronska-Kozak B. Scarless skin wound healing in FOXN1 deficient (nude) mice is associated with distinctive matrix metalloproteinase expression. *Matrix Biol.* 30(4), 290–300 (2011).

54 Bedair H, Liu TT, Kaar JL *et al.* Matrix metalloproteinase-1 therapy improves muscle healing. *J. Appl. Physiol.* 102(6), 2338–2345 (2007).

55 Mu X, Bellayr I, Pan H, Choi Y, Li Y. Regeneration of soft tissues is promoted by MMP1 treatment after digit amputation in mice. *PLoS ONE* 8(3), e59105 (2013).

• **Results from this study indicated that scarless wound healing is beneficial to digit regeneration after amputation in mammals and that MMP-1 can accelerate soft tissue regeneration without scar tissue formation.**

56 Mu X, Urso ML, Murray K, Fu F, Li Y. Relaxin regulates MMP expression and promotes satellite cell mobilization during muscle healing in both young and aged mice. *Am. J. Pathol.* 177(5), 2399–2410 (2010).

57 Samuel CS, Lekgabe ED, Mookerjee I. The effects of relaxin on extracellular matrix remodeling in health and fibrotic disease. *Adv. Exp. Med. Biol.* 612, 88–103 (2007).

58 Paz Z, Shoenfeld Y. Antifibrosis: to reverse the irreversible. *Clin. Rev. Allergy Immunol.* 38(2–3), 276–286 (2010).

59 Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. *Acta Biomater.* 5(1), 1–13 (2009).

60 Rosenwald M. A doctor, a pig, and a magical pixie dust that could regrow fingers. *Esquire*, 18th September (2007).

61 Cohen E. Woman's persistence pays off in regenerated fingertip. *Cable News Network*, 9th September (2010).

62 Muneoka K, Han M, Gardiner DM. Regrowing human limbs. *Sci. Am.* 298(4), 56–63 (2008).

63 Agrawal V, Johnson SA, Reing J *et al.* Epimorphic regeneration approach to tissue replacement in adult mammals. *Proc. Natl Acad. Sci. USA* 107(8), 3351–3355 (2010).

64 Agrawal V, Tottey S, Johnson SA, Freund JM, Siu BF, Badylak SF. Recruitment of progenitor cells by an extracellular matrix cryptic peptide in a mouse model of digit amputation. *Tissue Eng. Part A* 17(19–20), 2435–2443 (2011).

65 Ott HC, Matthiesen TS, Goh SK *et al.* Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat. Med.* 14(2), 213–221 (2008).

• **Demonstrates technical success at engineering a bioartificial heart using decellularization of the whole heart as a construct to provide architecture, the population of such a construct with an appropriate cell composition (with cardiac or endothelial cells) and maturation of this construct to develop nascent pump function by coronary perfusion in a bioreactor that simulated cardiac physiology.**

66 Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat. Biotechnol.* 23(1), 47–55 (2005).

67 Landis WJ, Jacquet R, Hillyer J *et al.* Design and assessment of a tissue-engineered model of human phalanges and a small joint. *Orthod. Craniofac. Res.* 8(4), 303–312 (2005).

68 Sedrakyan S, Zhou ZY, Perin L, Leach K, Mooney D, Kim TH. Tissue engineering of a small hand phalanx with a porously casted polylactic acid–polyglycolic acid copolymer. *Tissue Eng.* 12(9), 2675–2683 (2006).

69 Weinand C, Gupta R, Weinberg E *et al.* Toward regenerating a human thumb *in situ*. *Tissue Eng. Part A* 15(9), 2605–2615 (2009).

70 Messerli MA, Graham DM. Extracellular electrical fields direct wound healing and regeneration. *Biol. Bull.* 221(1), 79–92 (2011).

71 Gardner SE, Frantz RA, Schmidt FL. Effect of electrical stimulation on chronic wound healing: a meta-analysis. *Wound Repair Regen.* 7(6), 495–503 (1999).

72 Fehlings MG, Tator CH, Linden RD. The effect of direct-current field on recovery from experimental spinal cord injury. *J. Neurosurg.* 68(5), 781–792 (1988).

73 Borgens RB, Toombs JP, Breur G *et al.* An imposed oscillating electrical field improves the recovery of function in neurologically complete paraplegic dogs. *J. Neurotrauma* 16(7), 639–657 (1999).

74 Borgens RB, Blight AR, Mcginnis ME. Behavioral recovery induced by applied electric fields after spinal cord hemisection in guinea pig. *Science* 238(4825), 366–369 (1987).

75 Shapiro S, Borgens R, Pascuzzi R *et al.* Oscillating field stimulation for complete spinal cord injury in humans: a Phase 1 trial. *J. Neurosurg. Spine* 2(1), 3–10 (2005).

76 Ciombor DM, Aaron RK. The role of electrical stimulation in bone repair. *Foot Ankle Clin.* 10(4), 579–593, vii (2005).

77 Becker RO. Stimulation of partial limb regeneration in rats. *Nature* 235(5333), 109–111 (1972).

78 Sisken BF, Fowler I, Romm S. Response of amputated rat limbs to fetal nerve tissue implants and direct current. *J. Orthop. Res.* 2(2), 177–189 (1984).

79 Levin M. Bioelectric mechanisms in regeneration: unique aspects and future perspectives. *Semin. Cell Dev. Biol.* 20(5), 543–556 (2009).

80 Binggeli R, Weinstein RC. Membrane potentials and sodium channels: hypotheses for growth regulation and cancer formation based on changes in sodium channels and gap junctions. *J. Theor. Biol.* 123(4), 377–401 (1986).

81 Hotary KB, Robinson KR. Endogenous electrical currents and voltage gradients in *Xenopus* embryos and the consequences of their disruption. *Dev. Biol.* 166(2), 789–800 (1994).

82 Jenkins LS, Duerstock BS, Borgens RB. Reduction of the current of injury leaving the amputation inhibits limb regeneration in the red spotted newt. *Dev. Biol.* 178(2), 251–262 (1996).

83 Atkinson DL, Stevenson TJ, Park EJ, Riedy MD, Milash B, Odelberg SJ. Cellular electroporation induces dedifferentiation in intact newt limbs. *Dev. Biol.* 299(1), 257–271 (2006).

84 Han M, Yang X, Lee J, Allan CH, Muneoka K. Development and regeneration of the neonatal digit tip in mice. *Dev. Biol.* 315(1), 125–135 (2008).

• **Provided evidence that neonatal digit tip regeneration involves the formation of a blastema of proliferating cells expressing developmentally relevant genes and the differentiation of bone tissue by direct ossification.**

85 Ide H. Bone pattern formation in mouse limbs after amputation at the forearm level. *Dev. Dyn.* 241(3), 435–441 (2012).

86 Yu L, Han M, Yan M, Lee EC, Lee J, Muneoka K. BMP signaling induces digit regeneration in neonatal mice. *Development* 137(4), 551–559 (2010).

87 Paino F, Ricci G, De Rosa A *et al.* Ecto-mesenchymal stem cells from dental pulp are committed to differentiate into active melanocytes. *Eur. Cell. Mater.* 20, 295–305 (2010).

88 D'Aquino R, De Rosa A, Lanza V *et al.* Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *Eur. Cell. Mater.* 18, 75–83 (2009).

89 Giuliani A, Manescu A, Langer M *et al.* Three years after transplants in human mandibles, histological and in-line holotomography revealed that stem cells regenerated a compact rather than a spongy bone: biological and clinical implications. *Stem Cells Transl. Med.* 2(4), 316–324 (2013).

90 Ishihara A, Bertone AL. Cell-mediated and direct gene therapy for bone regeneration. *Expert Opin. Biol. Ther.* 12(4), 411–423 (2012).

91 Kang Q, Sun MH, Cheng H *et al.* Characterization of the distinct orthotopic bone-forming activity of 14 BMPs using recombinant adenovirus-mediated gene delivery. *Gene Ther.* 11(17), 1312–1320 (2004).

92 Li JZ, Li H, Sasaki T *et al.* Osteogenic potential of five different recombinant human bone morphogenetic protein adenoviral vectors in the rat. *Gene Ther.* 10(20), 1735–1743 (2003).

93 You Z, Bi X, Fan X, Wang Y. A functional polymer designed for bone tissue engineering. *Acta Biomater.* 8(2), 502–510 (2012).

94 Holzwarth JM, Ma PX. Biomimetic nanofibrous scaffolds for bone tissue engineering. *Biomaterials* 32(36), 9622–9629 (2011).

95 Bergman K, Engstrand T, Hilborn J, Ossipov D, Piskounova S, Bowden T. Injectable cell-free template for bone-tissue formation. *J. Biomed. Mater. Res. A* 91(4), 1111–1118 (2009).

96 Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature* 453(7193), 314–321 (2008).

97 Li Y, Negishi S, Sakamoto M, Usas A, Huard J. The use of relaxin improves healing in injured muscle. *Ann. NY Acad. Sci.* 1041, 395–397 (2005).

98 Negishi S, Li Y, Usas A, Fu FH, Huard J. The effect of relaxin treatment on skeletal muscle injuries. *Am. J. Sports Med.* 33(12), 1816–1824 (2005).

99 Mcgann CJ, Odelberg SJ, Keating MT. Mammalian myotube dedifferentiation induced by newt regeneration extract. *Proc. Natl Acad. Sci. USA* 98(24), 13699–13704 (2001).

100 Repesh LA, Oberpriller JC. Scanning electron microscopy of epidermal cell migration in wound healing during limb regeneration in the adult newt, *Notophthalmus viridescens*. *Am. J. Anat.* 151(4), 539–555 (1978).

101 Carlson MR, Bryant SV, Gardiner DM. Expression of Msx-2 during development, regeneration, and wound healing in axolotl limbs. *J. Exp. Zool.* 282(6), 715–723 (1998).

102 Gardiner DM, Holmes LB. Hypothesis: terminal transverse limb defects with "nubbins" represent a regenerative process during limb development in human fetuses. *Birth Defects Res. A Clin. Mol. Teratol.* 94(3), 129–133 (2012).