Do Humans Possess the Capability to Regenerate?

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Abstract

Urodele amphibians, including newts and salamanders, are amongst the most commonly studied research models for regeneration. The ability to regenerate, however, is not limited to amphibians, and the regenerative process has been observed in mammals as well. This paper discusses methods by which amphibians and mammals regenerate to lend insights into human regenerative mechanisms and regenerative potential. A focus is placed on the urodele and murine digit tip models, both of which share critical regenerative stages including wound healing, histolysis, and blastema formation. Formation of the blastema proved to be a crucial process necessary for regeneration, and is responsible for dedifferentiation and pattern formation. Additionally, the necessity of nerves, macrophages, and upregulation of several genes are discussed. The use of cellular therapy and development of extracellular templates shows promising opportunities in the fields of regenerative medicine and tissue engineering for the stimulation of endogenous repair.

Introduction

Organ deterioration can be caused by disease, damage, or aging. The shortage of viable organs for transplantation has grown severe; an average of twenty people die each day waiting for an organ. (www.unos.org) Additionally, there are nearly 2 million people with amputations in the United States alone, with limb loss caused by various reasons including vascular disease, trauma, and cancer. (www.amputee-coaliton.org) If a human organ or limb could be stimulated to regenerate it would radically change the quality of life and prognosis of many people.

There are two major classes of regeneration. Regular replenishment of a specific cell type or various types of cells within a tissue is defined as homeostatic regeneration. The mammalian epidermis is continuously undergoing homeostatic regeneration by the shedding and renewal of the epidermal cells. However, when complex tissue is replaced due to a wound this is referred to as reparative regeneration. Differentiation between these two types of regeneration is necessary; it expresses that regeneration requires coordination between multiple cell types, and that single cell level regeneration does not mean regeneration for complex tissue. For example, if an injury causes the loss of the epidermis and the underlying dermis, the skin will replace the lost tissue, but hair follicles derived from the epidermis fail to regenerate.(Simkin, Seifert, 2017) Using other models of reparative regeneration as a guideline, the mechanism by which humans may regenerate can be better understood.

Methods

This study was completed through the use of databases such as Touro College Library and Google Scholar. The National Institute of Health and NCBI were also used to find peer reviewed articles related to both amphibian and mammalian regeneration and regenerative mechanisms.

Urodele Limb Regeneration

Amphibians of the order Urodela, which include salamanders and newts, are the only tetrapod vertebrates capable of repeatedly undergoing regeneration of entire limbs. (Stocum, 2017) The mechanism by which they regenerate has been studied for over a century, with original studies focused on the histology and morphology of a regenerating limb, and more recent work on manipulating the regenerative process. The mechanisms by which they regenerate have been divided into several phases:

Wound Healing

Within a few hours after sustaining an injury, an enzyme catalyzed blood clot forms, sealing the open wound. Cells from the basal layer of the epidermis detach from the basement membrane and begin to travel towards the injury site. The basal cells relocate in thin sheets which begin to proliferate only once the wound is fully covered, forming a protective layer called the wound epidermis. The wound epidermis (WE) insulates the wound, protecting it from further external damage as well as providing a regenerative microenvironment. WE can grow up to 15 cell layers thick, ultimately forming the apical epithelial cap (AEC). (Campbell, Crews, 2008)

The breakdown of the extracellular matrix of the stump tissue through matrix metalloproteinases (MMP) and other hydrolytic enzymes is called histolysis. Degradation of the extracellular matrix by MMP is directly correlated with the rapid production and release of cells from the limb tissue. The significance of MMP's role was noted when an amputated limb was treated with the matrix metalloproteinase inhibitor GM6001 and the blastema failed to form. In addition to ECM remodeling, MMPs ensure that the basement membrane does not reform. This allows the wound epidermis and the tissues below to be in direct contact, which is necessary for the successful establishment of apical epithelial cap. Matrix metalloproteinases are released up until the developing limb bud is about medium sized, slowing only once inhibitory molecules such as TIMP1 are upregulated. (Vinarsky et.al. 2005)

Blastema

Two to five days post-amputation, cells from the internal tissues adjacent to the limb stump undergo dedifferentiation, forming a heterogeneous mixture of multipotent stem cells and limited progenitor cells. (Wallace et al., 1974) This accumulation of cells is called the blastema, one of the most fundamental stages in the regenerative response. Dedifferentiation is a nuclear reprogramming which prevents transcription of differentiation genes, and reverts cells into an embryonic-like state with greater developmental potential. While cells dedifferentiate during

blastema formation, they retain a form of cell memory, and are predominately restricted to the original cell phenotype. For example, cartilage and muscle tissue originating from the blastema are generally derived from cells with chondrocyte and myocyte lineage, respectively. Dermal cells were the exception, since they possess the ability to form other tissues in addition to the expected dermal tissue. (Tweedel, 2010) The cells are also known to possess a positional memory, specifically found in fibroblast derived blastema cells. Cells adjacent to neighboring cells with disparate positional identities are detected and then stimulated to regenerate the missing cells specific to the amputated structure. (McCusker et. al., 2015)

Macrophage count is significantly higher during blastema formation. Enriched numbers of macrophages reduce inflammation and clear cellular debris while stimulating angiogenesis, fibroblast migration, and production of MMPs. Amputated limbs depleted of macrophages during blastema formation led to scarring of the limb stump. On the other hand, depletion of macrophages after the blastema already entered the growth phase only caused regenerative delay. Dermal scar tissue separates the WE from the tissues below, so while the wound can close , the AEC is unable to form, thereby preventing the regenerative process from occurring. Macrophages are therefore essential in the early stages of blastema formation and influence the final outcome of a regenerating limb. (Godwin et. al., 2013)

The gene activity present in a regenerating limb is only partially known. Several genes upregulated during blastema formation include Msx1, Msx2, Nrad, Rfrng, and Notch. These genes have been linked to myogenesis inhibition, reduction of muscle regulatory proteins, muscle dedifferentiation, and stem cell self-renewal. Two of these genes, Msx1 and Msx2 , are specifically known to be expressed in the apical mesenchyme of all tetrapod vertebrates. Following amputation or a simple wound, Msx2 is expressed in the damaged epidermis and the surrounding tissue, while Msx1 is limited to the blastemal cells. Studies show that MSX genes affect the early regenerative stages, and in the absence of these genes tissue growth and differentiation is reduced. (Stocum, 2017)

Patterning

There are currently two major models for the study of pattern formation of a developing limb. The polar coordinate model is a one which presents a view on tissue regrowth and how patterning is induced. Cells are thought to have different tissue regrowth patterns based on their positional information or their particular anterior- posterior and proximal- distal sequence. This model also predicts that supernumeraries will only be induced when an ipsilateral graft is performed at angles of 180°. (Maden, Turner, 1978)

Cells with a specific differentiated phenotype may not be equivalent in terms of positional identity. This fact was proven through several studies where skin taken from the anterior portion of the same limb was grafted in strips, and the homotopic skin grafting led to regeneration. Heterotopic grafting, however, resulted in supernumerary body parts or regeneration failure. (Holder, 1981) Studies involving marked grafts provide direct evidence for the contribution of different tissues to limb tissue regrowth. Cells were proposed to possess the property of intercalation, in which growth is specific to their position. When two cells which are typically non-adjacent come in contact due to causes such as an injury or grafting, the cells will begin to divide. Division begins to replace all intermediary cells, and will terminate once all positional disparities between the two cells are resolved. When there is more than one possible route, intercalation will occur via the shortest path. (Bryant, Gardiner , 1987) This model was tested by placing two typically non-adjacent cells within a limb in contact with each other. Both cells acted via the intercalation response predicted by the polar coordinate model, proving these cells do indeed possess positional information. After the initial cells began pattern formation, the surrounding cells react secondarily to these cells, not necessarily through intercalation. (Holder, 1981) While cells of the regenerating limb were proven to possess positional information, the specific tissue containing this information was unknown. It was known, however, that the blastemal cells are derived from the tissues adjacent to the wound epidermis. Experiments involved with repositioning of the epidermis relative to other tissues of the limb did not have any effect on formation of the limb, nor lead to supernumerary structures. Similar studies done on nerve sheaths and skeletal muscle had the same results, leading to the conclusion that the epidermis, nerve sheaths, and skeletal muscle are not directly related to limb pattering. However, when even small grafts of skin was reoriented in the stump it led to supernumerary structures. Two of the tissue layers of the skin are the dermis and the epidermis. Since the epidermis was proven not to possess positional information, the dermis was determined to be the tissue layer containing the cells responsible for patterning in limbs. The dermis consists of mainly pigment cells and fibroblasts , and since tissue regrowth can occur in limbs without pigmented cells, fibroblasts were determined to be the cells possessing the positional information. It is thought that these fibroblasts form the patterned template for the limb, and the endothelium, myoblasts, nerves, and other cells use this information secondarily to complete limb regeneration. (Bryant, Gardiner, 1987) The polar coordinate model helps explain the way cells interact before and during regeneration, as well as how a specific pattern in a regenerating limb is induced. However, later experiments could not be explained fully with the polar coordinate model. For example, a supernumerary limb developed after an ipsilateral 90° rotation. (Maden, Turner, 1978)

The second model, the Boundary model, states that patterning in secondary embryonic fields is triggered by the boundaries

of cells of different determination. This model proposes testable predictions of the handedness, or the specific asymmetry in relation to the central axes, of supernumeraries with ipsilateral rotation. One prediction states that a supernumerary growing out of the anterior region of the host will possess the handedness of the host, while a supernumerary growing from the posterior side will have the opposite of the host. However, when the dorsoventral polarity of a supernumerary changes, (with normal anterior-posterior polarity) the posterior region will also have the handedness of the host.

A comprehensive study on supernumerary limbs found that the predictions proposed by the boundary model were accurate, with one exception. A single supernumerary growing from the posterior region of a host (possessing non-changing DV polarity) developed host handiness, instead of the opposite handiness that was predicted. The opposite handedness proposed only occurs when a supernumerary is one of a pair. (Meinhardt, 1983) The PCM and Boundary models together give an encompassing understanding of pattern formation occurs in regenerating limbs.

Retinoic Acid

Retinoic acid , also known as Tretinoin, is a lipophilic molecule possessing the capability to regenerate tissues and entire limbs of urodele amphibians. Retinoic acid (RA) is derived from Vitamin A, and is synthesized in the cell through several enzymes. Once formed, it functions to influence gene expression. RA binds to one of the two classes of ligand activated nuclear transcription factors, either the retinoid X receptors (RXR's) or the retinoic acid receptors (RARs). These receptors act as heterodimers on upstream DNA sequences of RA responsive- genes.

The effect of retinoic acid on regeneration varies based on concentration. In several clinical studies, transection done through the radius and ulna of axolotls caused the radius, ulna, carpals, and digits to regenerate. (Fig. 1, A) However, when a low dosage of retinoids was applied to the amputation, an entire radius and ulna regrew in tandem with the original. (Fig. 1, B) Raising the dosage further led to regeneration of an extra elbow joint (Fig. 1, C), and with a higher concentration still, the limb was completely regenerated. (Fig. 1, D, E) (Maden, Hind, 2003)

Additional regeneration effects were found in a study done on the common frog, Rana Temporaria. When treated with high concentrations of retinoids, not only did the complete foot regenerate , (Fig. 1, F, G) but sets of limbs-including the bony pelvis, were regenerated as well. (Fig. 1, H) The first studies done show how retinoids, including retinoic acid, reduplicated the proximodistal axis. Since the pairs of limbs were mirror images of each other, it is clear the dorsoventral and anteroposterior axes had been reduplicated as well. In addition to regenerating limbs from preexisting cells, RA can alter the course of lineage specific cells, and form different structures entirely. For example,

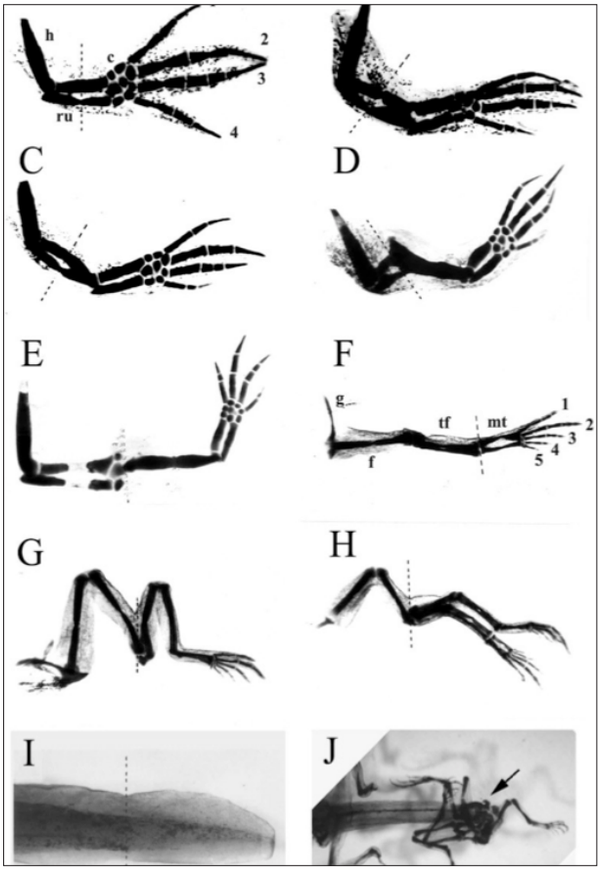

Figure 1 Treatment of an amputated limb with a high retinoid concentration regenerated the complete limb, and in some cases even pairs of limbs, from the amputation plane. (Maden, Hind, 2003)

the tail of a tadpole from the species Uperdon systoma and Rana temporaria can regenerate completely after amputation. (Fig. 1, I). However, treatment with RA led to the development of multiple hindlimbs, instead of the expected tail. (Fig. 1, J) In addition to retinoic acid's direct relationship with regeneration in amphibian limbs, it has a strong effect on mammalian regeneration, which will be discussed later in this text.

Mouse Digit Tip Regeneration: Mammalian Model

Mice have the capability to regrow epidermis, nail, bone, and other structures of the digit. (Simkin, et. al., 2013) The process by which regeneration occurs for the murine digit is similar to the human response, and has become a primary resource for the study of human regenerative potential.

The response in mice is dependent on the region of amputation. If a wound occurs at the outermost tip of the third phalanx bone (P3) the wound will heal in a regenerative manner. However, if an amputation take place at the proximal region of the P3 bone, or the second phalanx bone (P2), the wound will develop into a scar. These two distinct areas serves as a

guideline for understanding the different processes of scar formation and epimorphic regeneration.

Wound Healing

The immediate response to an injury at the digit tip is similar for both the P2 and P3 bone regions. In the initial stage, inflammation, a blood clot is formed and the blood vessels dilate, allowing inflammatory cells, neutrophils, and other necessary molecules to pass to the wound site. Wound closing time ranges from a few hours to over a week, with fetal mice recovering the fastest. This wound healing process is distinctly longer than the wound healing process occurring for amphibians. In the second stage, histolysis, the divergence between the regenerative-competent and regenerative incompetent region becomes apparent. In the regenerative region, the breakdown of tissue from histolysis causes the bone to completely detach about one week post amputation. The detached bone will fall off due to the accumulation of epidermal cells beneath it, thereby effectively sealing the wound. Amputations at the P2 and the proximal P3 regions undergo histolysis as well, but to a lesser extent and generally the digit remains attached. Unlike the P3 bone, in the regenerative-incompetent region collagen deposits between the tissue layers, inhibiting communication and ultimately leading to the development of a scar. (Quijano et. al., 2016)

The developmental genes Msx1 and Msx2 are found in regenerating digits and are believed to regulate the expression of bone morphogenetic proteins (BMPs). These proteins are necessary for cartilage and bone repair and have been identified as powerful proliferative molecules. Amputated digits treated with the BMP-inhibitor noggin did not only lose their ability to proliferate, but were unable to regenerate as well. (Manjong et. al., 2003) Several of these proteins including Bm7, Bmp2, Bmp4, and their receptors, Bmpr1a and Bmpr1 b, are expressed in specific regions of the phalanx and communicate via signaling. The regenerative failure seen at the proximal region of the P3 bone has been linked to an absence of BMP signaling. The bone stump of a control digit amputated at the proximal level elongated slightly but ultimately failed to regenerate. However, treating amputations at the proximal P3 region with one BMP bead led to a regenerative response. Specifically, signaling by BMP7 stimulated the greatest elongation of the terminal phalangeal, and caused an overall skeletal elongation of 58%. Two weeks post injury a regenerated digit was integrated within the stump bone. While the bone formed is histologically distinct from the stump bone, the terminal phalanx is still successfully restored.

A small hole connecting the marrow region with the dermis is formed at the ventrolateral area of the P3 bone when there is a severance at the more proximal region of the bone. However, digits regenerated from implanted BMP beads do not completely seal this hole. This can serve as a marker that amputation did occur at the proximal level and not from discrepancies of severance level. Introduction of one BMP bead caused entire tissues to regenerate at a typically non-regenerative region. This data indicates that the cells necessary for a regenerative response are present, it is the microenvironment which is responsible for regenerative failure. (Yu et. al., 2010)

Neural Requirement and the AEC

Nerves play a key role in the regenerative process, and often rely on neurotrophic factors for their maintenance. Neurotrophic factors are biomolecules which encourage neural survival and growth, and have been found necessary for both immature and fully developed nerves. One of these biomolecules, the Nerve Growth Factor (NGF), discovered in the early 1950s, has proved particularly important in regeneration of the peripheral nervous system. Several experiments found that NGF-treated nerves were able to regrow damaged nerve cells that would have otherwise been lost. (Rich et. al., 1989) Once a blastema has reached the medium bud stage it no longer requires nerves for differentiation or morphogenesis, but remains nerve dependent for proliferation. Nerves are also involved with transcription; denervation led to a decrease in RNA production by over 73%. (Stocum, 2017)

The AEC plays an equally important role in regeneration. Scar tissue forms on an amputated limb of a urodele when the AEC is taken out multiple times. When the apical epithelial cap was removed from the distal portion of newt's forelimbs, both DNA synthetic activity and overall mitotic index decreased, suggesting the AEC plays a mitogenic role in the regenerating wound. (Globus, 1980)

Nerves and the apical epithelial cap both play fundamental roles in regeneration, suggesting some relationship between the two. One hypothesis is that the nerves and AEC play separate yet synergistic roles in the cell cycle. This idea was formed from the study of labeled blastema cells within an amputation. If either the nerves or wound epidermis were removed, the cells were apprehended in the second growth phase of the cell cycle, ultimately undergoing apoptosis or removal through macrophages. However, if the AEC was re-innervated, mitosis in the blastema increased as much as 10-fold. (Mescher et. al., 2000) This mitotic increase only occurred if both nerves and wound epidermis were present. It is thought that the blastema cells are stimulated by a wound to enter the cell cycle, and nerves send mitogenic signals to the undifferentiated cells maintained by the AEC. (Stocum, 2017) Furthermore, the AEC is believed to contain all factors for the cell cycle, yet depends on nerves to express them. It was found that any neural requirements for regeneration are only acquired during late regenerative stages, and with aneurogenic limbs of urodeles, in which the neural tube was extracted during embryogenesis, there is no neural dependency developed at all. (Brockes, 1987)

A final relationship between nerves and the AEC is that they

both possess the same mitogen, neuregulin 1 (NRG1). Studies show that transcripts of neuregulin 1 are expressed by over half of the blastema mesenchyme cells and by the basal cells of the AEC, while immunostaining techniques revealed the presence of neuregulin 1 in both peripheral limb nerves and dorsal root ganglia. Amputated innervated limbs treated with mubritinib, an inhibitor which prevents signaling by neuregulin 1, were unable to form the blastema. When 16-day innervated blastemas were treated with mubritinib it resulted in the formation of miniature regenerates, identical to the regenerates observed when denervation of a blastema is postponed until a stable quantity of cells have formed. The nerves present were enough to stimulate a partial regenerative response. However, the blastema is also stimulated when NRG1-soaked beads were placed 7 days post-amputation under the wound epithelium of denervated limbs. Implanting beads every four days starting from about 3 to 5 weeks post-amputation allowed some regeneration, however not as much as limbs which were innervated. (Stocum, 2017) These studies indicate the blastema cells produce neuregulin 1 independently of nerves, but in a quantity not great enough to initiate mitosis. Sensory and motors neurons act by inducing both the basal cells and the blastema cells of the apical epithelial cap to increase production of neuregulin 1 to levels required for mitosis. This finding also explains the previously mentioned 10-fold increase of blastema cell production when the AEC is re-innervated.

Blastema Formation

The blastema formed during mouse digit tip regeneration has similarities to the blastema formed in the regenerating urodele. Beginning from 12 hours post amputation for fetal mice to 12 days post amputation in the adult model, cells begin to aggregate at the outer portion of the amputation stump. The cells of the blastema stem from neighboring tissue, are avascular, and proliferate at a significantly faster rate than cells in nonregenerating tissue. Studies show that the cells of the blastema are lineage restricted. It was found that mesodermal cells will form tissues of the dermis, blood vessels, tendons, and the mesenchyme of the digit, but will not participate in the formation of ectodermal cells such as the epidermis, sweat glands, and nail plate. Likewise, ectodermal cells were found to contribute solely to the formation of ectodermal tissue. (Quijano et. al., 2016)

Similar to the urodele model, retinoic acid has regenerative effects on mammals as well. The most advanced clinical study to date was the use of retinoic acid to induce alveolar regeneration in rats and mice. RALDH1, an enzyme responsible for the synthesis of RA, is present in a specific spatial arrangement from postnatal day 1 (PND1) to PND4 in a manner correlating with the patterning of alveolar proliferation. This enzyme is expressed in alveolar parenchyma from PND4, where there is a sharp increase of cell proliferation. When an inhibitor was used to prevent RA production, air spaces began to develop, the diameter of the alveoli increased, and their overall formation was delayed. RA receptors (RARs) and its isoforms were present in initial alveolar formation, with specific isoforms (RARγ) acting as stimulants for alveolar development and others (RARβ) serving as negative regulators. Based on these findings it was hypothesized that RA is needed for the primary stages of alveolar development, and can stimulate a regenerative response. A study on the lungs of adult rats with induced alveolar loss proved this indeed was the case. Treatment of these lungs with retinoic acid restored all normal lung parameters within 12 days. A similar test on mice treated with RA from PND30 to PN42 and then grown to PN90 showed complete regeneration of alveolar tissue. From this data, it was it was deduced that RA reactivates the gene pathways used during embryonic alveolar formation. (Maden, Hind, 2003)

Tissue Remodeling

During the tissue regrowth process, the new tissues of the regenerate are formed. The deposition of soft connective tissue also takes place during the scar forming process and therefore cannot be used as a definitive indicator for the beginning of regeneration. Regeneration of bone, however, is unique to the regenerative process and serves as an indication that regeneration has begun.

Differentiation of regenerated bone begins at the area closest to the body and progresses outwards until the digit tip. As the digit tip lengthens, new bone is added through direct ossification, forming woven or fibrous bone. The regenerated bone is porous, less dense, and therefore weaker than cortical bone.

Furthermore, the woven bone will have a significantly larger volume than the original cortical bone, while still retaining a constant length. Eventually, the pores of the regenerated bone will shrink in size, and the density will increase, yet the regenerate will still remain histologically distinct.(Simkin et. al. 2015)

While the microarchitecture of the original and regenerated digit may differ, many qualities of the original is retained. For example, in several studies the dorsal bend of the nail, the area the ventral fat was situated, and the tapering formation of the digit were restored.

Human Regeneration

Regular cell turnover and renewal occurs in many organs and tissues of the human body, including the cells of the intestinal epithelium, stomach, and epidermis. (Miguel et. al. 2017) While complete regeneration of complex tissue is unusual, reports of distal phalanx regeneration have been documented for both children and adults. In one early case, an infected adult fingertip was amputated and then treated with frequent dressing changes. Three months later, X-ray imaging showed the fingertip had completely regenerated. (Dolan et. al. 2018) Since then there has been

numerous clinical reports on fingertip regeneration, all sealed with tight bandaging or direct suturing, which is thought to create a microenvironment conducive to a regenerative response.

Tissue Engineering and Regenerative Medicine

The fields of tissue engineering and regenerative medicine are dedicated to finding methods to restore both the structure and functions of damaged organs and limbs. (Shieh, Cheng , 2015) There are two central strategies used to obtain this goal. The first is the use of cellular therapies, such as iPSCs and MSCs. For example, a recent study made the use of bone marrow-derived mesenchymal stem cells (MSC-CM) in attempt to regenerate bone. MSC-CM contains cytokines such as IGF-1, transforming growth factor (TGF-β1), and vascular endothelial growth factor, and was found to increase the development of new blood vessels and cell proliferation and mobilization. In a clinical study done to test the effectiveness and safety of these stem cells, a cavity in the mouth of patients requiring sinus floor elevation surgery or guided bone regeneration surgery was filled with MSC-CM. Six months post-surgery, new bone was present for all the participants.

The typical method for repairing extensive bone damage is through a bone graft. Autogenous bone grafting is osteoinductive, osteoconductive, and osteogenic and is therefore considered the highest standard available when grafting bone. However, the procedure requires retrieving bone directly from the patient, and is painful, expensive, and associated with higher rates of morbidity. (Katagiri et al. 2016) MSC-M is currently undergoing more comprehensive clinical studies and may prove to be a better alternative.

A second a major goal is to transition damaged tissue into a blastema in attempt to promote differentiation and regeneration. Some tissues, including the epidermis, heart, and lung, do not require formation of a blastema to regenerate. However, their microenvironment controls the cellular response to a wound and can therefore also initiate differentiation. One method used to help regulate this microenvironment and facilitate regeneration is the creation of biologic and synthetic scaffolds. One such scaffold, called the ECM scaffold, is formed by decellularization of an organ or tissue, thereby creating a meshwork of proteins in tissue-specific structure. The scaffold's composition will vary based on its source, since the ECM scaffold is composed of the molecules released by the resident stem cells of an individual organ. This biologic scaffold serves as a biochemical signaling platform and a template for the regenerating tissue, and provides both a macro and microenvironment which promotes functional tissue repair. The environment and the processes that occur in the presence of an ECM scaffold are similar to that of a normally developing tissue but are typically absent in the default repair process of an injured tissue. Work is currently being done to regenerate the pancreas, kidneys, and the muscles of the heart, with the greatest amount of success seen in regeneration of the bladder, skin, airway, and bone. (O'Brien, 2011) Studies on the effectiveness of engineered tissues have been promising; however, some of the testing done for scaffolds efficacy were not standardized processes. (Moreno et. al., 2016) and there is a need for a greater amount of clinical research with more consistent testing methods.

Conclusion

The challenge of stimulating regeneration is to create an environment which permits a regenerative rather than a fibrotic response to a wound. The fact that BMP signaling induced regeneration of the proximal P3 bone in mice shows this endeavor is already a possibility. The regenerative pathways of the urodele limb and murine digit tip serve as a solid basis for achieving this goal in humans. While their regenerative pathways were not identical, both culminate in the formation of the blastema and to complete tissue renewal. A focus in the fields of regenerative medicine and tissue engineering is to create a microenvironment that parallels the blastema, with the purpose of creating integrated multi-tissues for organ and limb formation. The use of synthetic and biologic scaffolds, as well as manipulation of stem cells already made significant steps in generating endogenous repair. Human regeneration once seemed an impossible goal, but current advancements and the regenerative success seen in human fingertips gives hope for regeneration of complete organs and limbs in the future.

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