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Limb regeneration in humans: Is it possible?

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Abstract
Limb regeneration is a concept that has been used in many popular movies, including the 2012 movie, *The Amazing Spiderman*, in which the villain, Dr. Connors, injects himself with lizard DNA with the purpose of regenerating his arm. Combining the DNA of a lizard with a human, however, will not ensure regenerative abilities in humans. This is a result of the signalling pathway, wnt signalling/ β-catenin, not being activated to express regenerative genes. Natural regenerative species, such as axolotls and tadpoles, have specific Hox genes responsible for regeneration of limbs after amputation, including *HoxA9, HoxA11*, and *HoxA13*. This paper discusses the possibility of introducing these Hox genes into human DNA by virus-mediated transfection as well as using an expression vector, thereby enabling limb regeneration. The wnt signalling pathway could be used to express the three Hox genes at the wound site to stimulate regeneration of the amputated limb.

Introduction
In many popular movies, the concept of human limb regeneration is often toyed with, especially in many superhero movies. In the 2012 movie, *The Amazing Spider Man* [1], the genius villain Dr Connors, also known as 'The Lizard', combines the DNA of a lizard with his own DNA to gain limb regeneration [2, 3]. In the movie not only does this allow Dr. Connors to regrow his arm, but also inadvertently changes his appearance to look more lizard-like.

There are a few species in nature that have the ability to regenerate a limb after it has been amputated, such as the axolotl (a type of salamander) and some tadpoles. This ability stems from their Hox genes; a family of genes concerned with the spatial development of segments of the body and limbs [4]. In this paper we will only consider the possibility of limb regeneration through gene expression by transfection of the Hox DNA, and not the possibility of looking more reptilian.

Combining Lizard and Human DNA
Combining lizard and human DNA has potential for being plausible, as lizards have a similar DNA make up to humans; they are the most closely related species to humans with regenerative abilities [5]. In theory, it would be possible to combine human and lizard DNA to attain regenerative properties. However, in practice this is not feasible, as different species express different genes. Due to this differential gene expression, even if the genes were combined, the traits would not be expressed as the expression pathways involved in limb regeneration would not necessarily be active in humans.

Hox Genes Involved in Limb Regeneration
The *HoxA* regulatory domain is the one of the domains of the gene family that is thought to have the most importance to limb development and alignment [4]. It has been shown that mice with no *HoxA* function in their forelimbs experience arrested development of those limbs [6]. Specific *HoxA* genes that have been identified to be involved in limb regeneration in axolotls are the *HoxA9, HoxA11,* and *HoxA13* genes, as shown in figure 1. In the axolotl these genes are expressed in the limb buds during foetal development, and in blastemas during limb regeneration [7]. Unlike in limb development, during limb regeneration these genes are expressed in the same population of cells, enabling regeneration regardless of the amount of amputation that occurs [7]. *HoxA11* and *HoxA13* genes are also expressed during limb regeneration in *Xenopus* tadpoles [8].

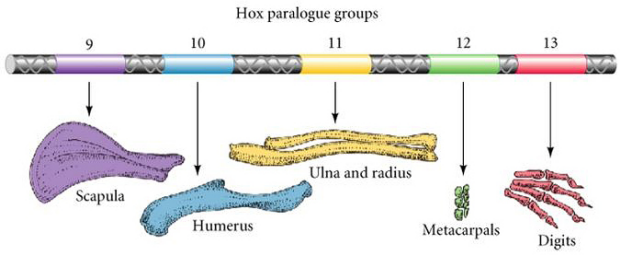

Figure 1 – Hox gene groups responsible for the development of different limbs [9].

Expressing *HoxA13* and *HoxA9* genes in humans.

In order to gain regenerative abilities in humans, the genes responsible for this trait need to be expressed and turned on. The wnt signalling/β-catenin signalling pathway is responsible for turning on genes in axolotls [10]. This pathway can be utilised in humans to express *HoxA9, HoxA11,* and *HoxA13* which would then be able to regenerate tissue in the amputated area. In the signalling pathway of the regeneration of amputated limbs of axolotls, active β-catenin is found to be present during regeneration [10]. Thus, β-catenin can be activated which would then activate the wnt signalling to express the genes needed for regeneration.

Limb regeneration involves a five-stage process; wound healing, dedifferentiation of cells, blastema formation, morphogenesis and growth [10]. In the initial process of the wound healing would have already occurred in humans of the amputated areas, thus this signalling pathway is activated to initiate the dedifferentiation of cells around the amputated region [10]. During wound healing, epithelial cells from an apical epidermal, cap around the wound. In this signalling pathway, the cells at the distal end of the cap of the amputated areas are activated and released which then proliferate to form a blastema. The cells then undergo morphogenesis and begin to dedifferentiation into the corresponding tissue needed for limb development. The limb will then continue to grow until it reaches the normal length for that limb [10]. For example, if the arm was amputated as in the movie, the arm will grow until it reaches normal length. As Hox genes are being expressed which are normally present in regeneration of the tail, the regeneration of arm with a hand and fingers would be more complex as it involves development of more segmentations. Thus, even if this were possible, it would be more like that the arm would form to normal length with a hand, but formation of individual fingers would be more complex.

An approach to achieve limb regeneration is through gene transfer by DNA transfection of the gene into cells in the amputated area [7]. As the axolotl *HoxA13* gene is identical to the human *HoxA13* gene [7] it could be extracted by an enzyme from a DNA vector and inserted into the DNA of cells at the wound sites [7]. In the same way, *HoxA11* and *HoxA13* would be injected into the site of the wound. Virus-mediated transfection or transduction can be used to achieve the insertion and expression of the *HoxA9, HoxA11,* and *HoxA13* genes *in vivo* in human cells [11]. The viral vector is injected into the amputated area to ensure the genes are delivered to cells in the area where regeneration is required. The viral vector, along with the transgene will release chemicals that activate the wnt signalling pathway, to activate regeneration of tissue. Another possibility to ensure expression of the two genes would be to inject an expression vector that contains regulatory sequences required for the genes [12].

In axolotls, the genes are not uniformly distributed amongst all of the cells at the wound site. For example, both *HoxA13* and *HoxA9* are only present in mesenchymal cells, and not in epithelial cells [8]. This has consequences for the artificial insertion of the DNA to the wound cells, as only the mesenchymal cells should get the DNA.

Conclusion

Whilst Dr. Connors combines his DNA with a lizard's DNA in the movie *The Amazing Spider Man*, this is not a feasible way of actually obtaining regenerative properties for limbs. Instead, the HOX-genes *HoxA9, HoxA11,* and *HoxA13* have been discussed as a possible method of limb regeneration by insertion of these genes into human DNA via DNA transfection. This is theoretically possible; a signalling pathway, such as the wnt signalling/β-catenin signalling pathway could be utilised to express the three relevant genes in the mesenchymal cells at the wound site, stimulating limb regeneration. However, even with the presence of these genes and an active signalling pathway, the complexity of the limb may not be reformed to its original structure.

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