# Summary of Responses to Request for Information (RFI): Seeking Rapid Advancement of Research on Limb Regeneration

**Notice Number:** NOT-HD-20-004

**Purpose of RFI:** NICHD seeks to facilitate and accelerate limb regeneration research with the goal of making substantial advances towards human limb regeneration within the next five years. This aligns with an aspirational goal from the NICHD Strategic Plan to "Advance the ability to regenerate human limbs by using emerging technologies to activate the body's own growth pathways and processes."

In order to understand both the current state of the limb regeneration science and the potential for rapid advancement, this RFI sought input from experts with knowledge of limb regeneration research.

**Number of Responses:** A total of 31 responses were received from individual researchers.

**Disclaimer:** This document summarizes the responses received to the afore mentioned RFI. It does not purport to review current literature or express the opinions of NICHD program staff.

**Summary of Responses:**

Parallel *in vitro* and *in vivo* approaches are proposed to achieve rapid progress in limb regeneration research. These include research on **endogenous tissue regeneration, *in vitro* and stem-cell based studies,** and **novel unconventional studies**. The responses emphasized multiprong approaches; comparative biology studies in model organisms (smaller invertebrates to mouse and pig), recapitulating embryonic development, generating embryonic limb bud-like cells from iPSCs, engineering artificial blastemas, as well as pursuing unconventional/novel ideas. Integration of data from various approaches is seen as key to gaining meaningful insights on how best to approach limb regeneration in humans. This can only be achieved through an exchange of ideas [workshop(s)] and collaborations between interdisciplinary team of scientists such as tissue engineers, developmental biologists, and stem cell biologists.

**Endogenous tissue regeneration** remains the focal point for accelerating limb regeneration research. Blastema formation is a hallmark of tissue regeneration and contrasts with fibrotic scarring in non-regenerating tissues. Several key areas were identified that require further investigation to generate a comprehensive knowledge of blastema biology. These include knowledge of regeneration-permissive and regeneration instructive signals, interaction between the wound epithelium and the underlying blastema, immunological responses and macrophages, nerve signals, tissue type specific progenitors, mesenchymal stem cells, cell/tissue dedifferentiation and genomic integrity in proliferating blastemas. Gaining rapid and broad insights into these biological processes will require the use of innovative approaches and state of the art technologies (scRNAseq, CHIPseq etc.) to study limb neogenesis in smaller, regenerative-competent invertebrates and vertebrates. It is suggested that big data approaches (mRNA, lncRNA, microRNA, epigenomics etc.) to comparative biology studies (invertebrates, vertebrates and mammals) are crucial for developing strategies to fully or partially restore lost limb tissues and functions in mammals including humans. As noted by one investigator, partial regeneration of a femur facilitates better prosthetic fitment and thus enables mobility and function.

The signaling molecules and pathways identified by the respondents as critical in the context of endogenous tissue regeneration include Leptin and reactive oxidative species-ROS (upstream signals triggering regenerative response), Hox genes (positional information, patterning), PAR2 (broad role in regeneration), TBRII+ and Prx1+ (progenitor cells), and EGFR (AP and DV patterning, AER formation, and cell survival).

***In vitro* and stem-cell based studies** were recognized as complementary approaches to the endogenous tissue regeneration. Generating iPS-derived embryonic limb bud-like cells (recapitulating embryonic development), stem cell transplantation, stem cell oxygenation and ischemic tissue regeneration, exogenously applied biological cues over time scales that recapitulate tissue development and regeneration are all recognized as potential areas of interest. Some respondents believe generating an artificial blastema is a key parallel approach to accelerating limb regeneration. It is conceivable that knowledge gained from research on endogenous regeneration is crucial in informing efforts to successfully engineer an artificial blastema and its microenvironment.

**Unconventional and radically different ideas** also need to be encouraged to invigorate the field. Examples of such ideas include investigation of the roles of metamorphosis hormones, endocrine function (e.g., thyroid function) and non-morphogenetic parameters such as biomechanical forces, sensorimotor activity, and metabolic parameters.

As noted by multiple respondents, common biological pathways/mechanisms might play essential roles in regeneration of multiple tissue types. It was proposed that studies of hair neogenesis and ear regeneration in the spiny mouse have the potential to inform aspects of limb regeneration. Zebrafish heart regeneration is viewed as a good model to determine how scars can be dissolved to potentially activate tissue regeneration.

It is clear that successful limb regeneration will require integrative approaches to regenerate all tissue types that constitute a functional limb; skeletal, muscle, ligament, joint, skin, vasculature and nerve. Another area of critical importance to limb regeneration is tissue scale, an essentially unexplored area. It is noted that Salamanders can serve as an excellent model to provide invaluable input on the topic since these animals initially regenerate disproportionally small limbs that grow rapidly to the appropriate (and functional) size.

Finally, resources and tools came across as critical needs for the research community. Examples of areas needing improvement include transcriptomic and genomic resources, real time imaging tools, spatial-temporal manipulation technologies (e.g., optogenetics), microCT (e.g., 3D assessment of bone regeneration), bone density and architecture assessment tools, and technologies to deliver cargo to awaken the latent regenerative machinery (such as viral vectors).

**Conclusion:**

The National Center for Medical Rehabilitation Research and the Developmental Biology and Structural Variation Branch of NICHD would like to thank all respondents for their thoughtful comments. This feedback will help to inform their deliberations related to facilitating and accelerating research in the field of limb regeneration.