Digit tip regeneration:

From mouse to man

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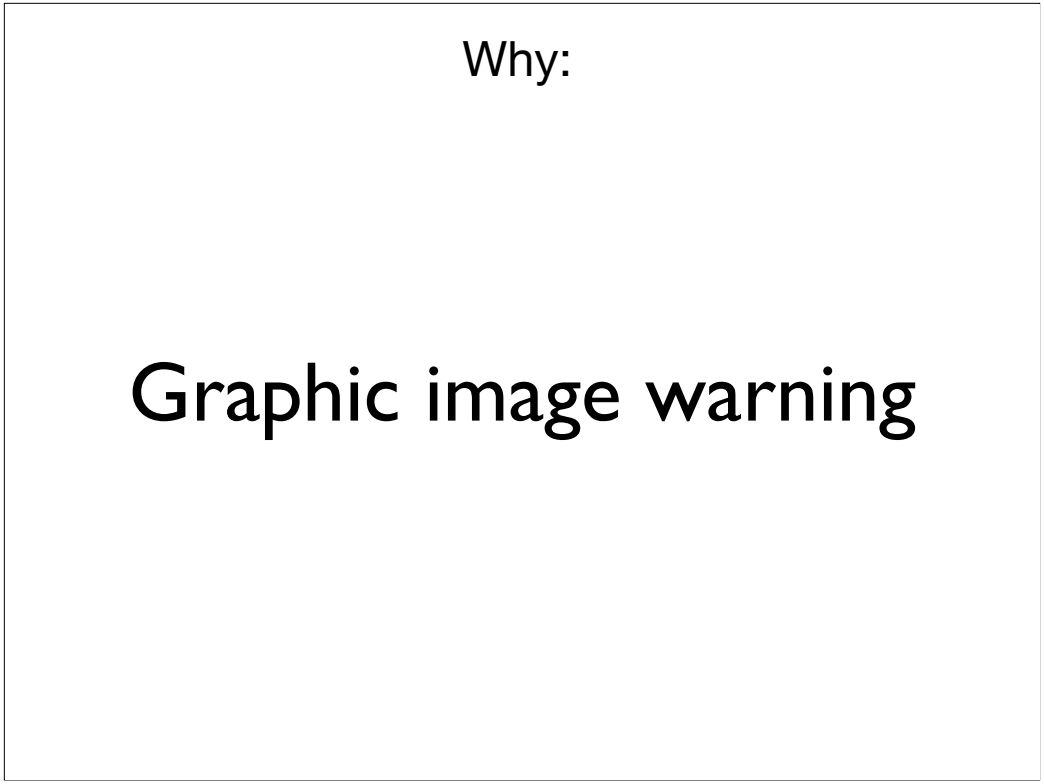
Musculoskeletal Systems Biology Lab
ISCRM Research Update
November 3, 2020

Hi I'm Chris Allan from the Musculoskeletal Systems biology lab headed by Ron Kwon.

Briefly:
-mouse
-human
-wound environment
-next expts/vision for future



I have a few clinical slides that I'll warn about ahead of time.

Why does digit tip regeneration matter?

Because it provides encouraging evidence that even adult human cells can participate in a regeneration response given the right environment.

Why:

Lawnmower injury to three digits; one treatment option might be to shorten the bone and close soft tissues over it, but this young woman was very opposed. We could try dressing changes and see what happens, but can these regrow?

Regeneration?

Perfect replacement of lost part

And if we can’t regenerate a digit tip we’re never going to regenerate a limb.

Let’s see what we can learn from other organisms.

The phenomenon of digit and limb regeneration attracts great interest because, for those species capable of it, it's a strategy that identically replaces lost parts.

Requirements:

1) progenitor cells (“regeneration-competent”)

2) patterning information

3) permissive environment

...can mammals do this? Can humans?

There are at least three hurdles to regrowing a lost part.

You need regeneration-competent cells that can build functional tissue after injury--not just scar, and there must be an accumulation of enough of those cells after amputation--a blastema--to rebuild the missing part.

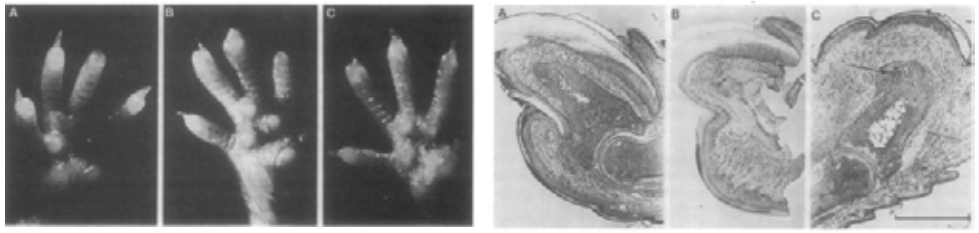
You need patterning information to direct the cells to make what was lost.

And you need a permissive environment--to protect the regrowing part, and maybe to exert some mechanical or other signals to stimulate the process.

Can adult humans do this?

Mice Regrow the Tips of Their Foretoes

Abstract. *Mice will replace the tip of a foretoe when it is amputated distal to the last interphalangeal joint. Amputation of the digit more proximal to the joint does not result in regrowth of the foretoe. Though this growth shares certain similarities with the epimorphic regeneration of amphibian limbs, the two processes are not the same. The regrowth reported here in mice is probably similar to the scattered clinical reports of fingertip regeneration in children, and presents a model system with which to explore the controls of wound healing and tissue reconstruction in mammals.*

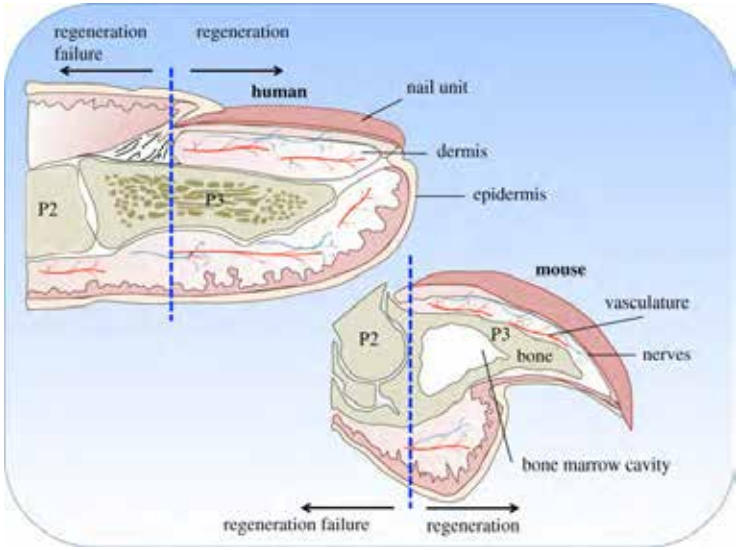


Borgens RB. Mice regrow the tips of their foretoes. *Science*. 1982;217(4561):747-750.

Maybe we can learn from another mammal, the mouse.

Mice do regrow amputated digit tips.

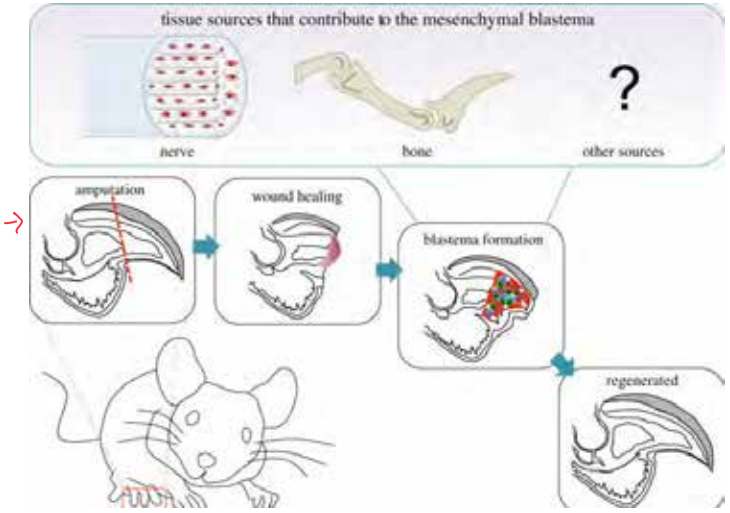
Digit tip comparative anatomy



Storer MA, Miller FD. 2020 Cellular and molecular mechanisms that regulate mammalian digit tip regeneration. Open Biol. 10: 200194.

Digit tip regeneration is a rare example of multi-tissue regeneration in mammals. The mouse digit tip is similar enough to human to make for a good model.

Regeneration = multi-stage process

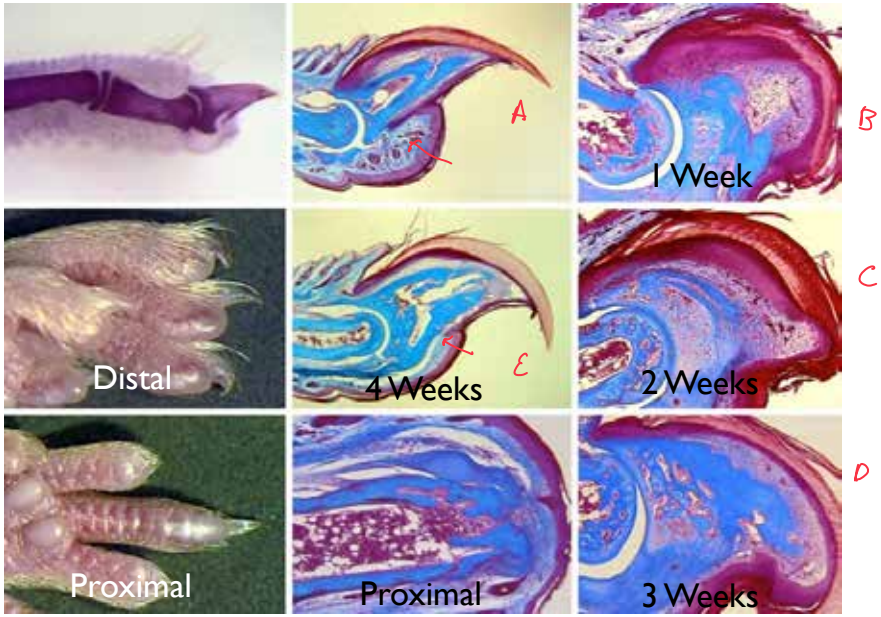


Storer MA, Miller FD. 2020 Cellular and molecular mechanisms that regulate mammalian digit tip regeneration. Open Biol. 10: 200194.

This is from a great recent review out of Freda Miller's lab showing the multi-step process.

The middle row shows the amputation plane, initial formation of the wound epidermis, blastema formation, and the final regrown digit tip.

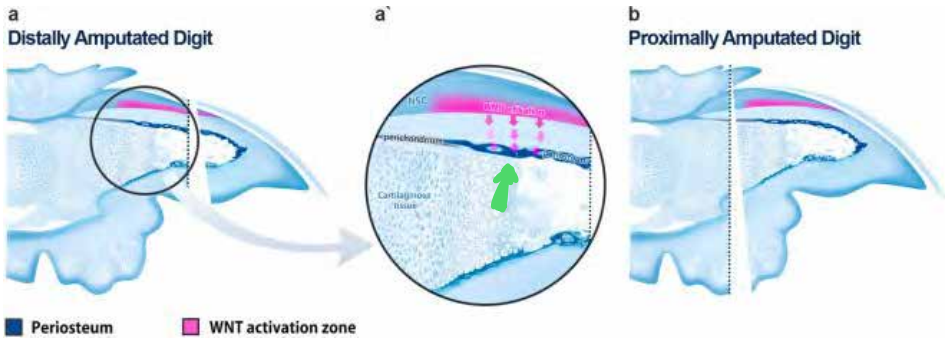
Mouse digit tip regeneration in vivo



Muller et al., 1999

This panel shows the process in stepwise fashion.

Level-dependent regenerative response



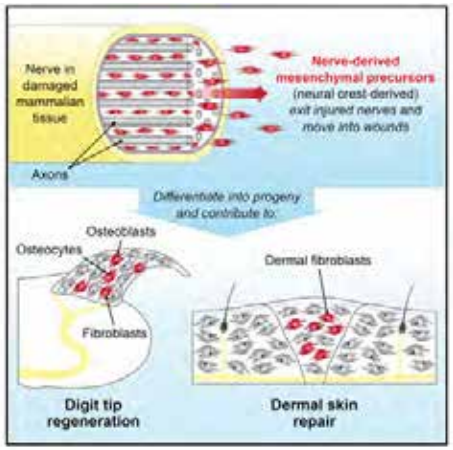
Sensiate LA, Marques-Souza H. Bone growth as the main determinant of mouse digit tip regeneration after amputation. Sci Rep. 2019 Jul 4;9(1):9720. doi: 10.1038/s41598-019-45521-4. PMID: 31273239; PMCID: PMC6609708.

You need some of the nailbed present for regeneration to happen. This is one proposed mechanism.

Wnt activation in the nail epithelium (shown in dark pink) signals to bone precursor periosteal cells, (shown in dark blue) directing appositional bone regeneration;

Proximal amputations remove both the signaling and the responding regions, so regeneration fails.

Nerve-derived mesenchymal precursor cells become bone, dermis

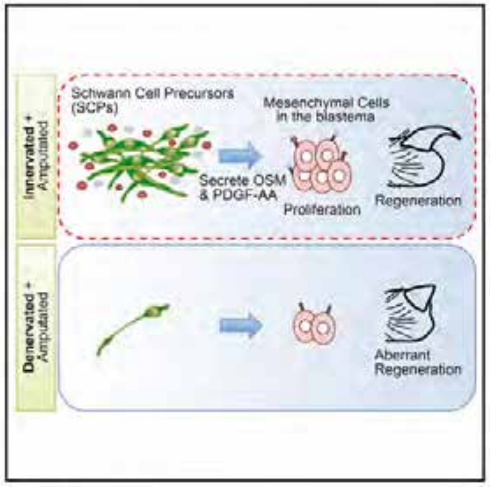


- Peripheral nerves contain four transcriptionally distinct mesenchymal populations
- Endoneurial *Pdgfra*-positive cells have mesenchymal precursor properties
- Nerve mesenchymal cells contribute to bone formation during digit tip regeneration
- Transplanted and endogenous nerve mesenchymal cells contribute to dermal repair

Carr et al., 2019, Cell Stem Cell 24, 240–256

Mouse digit tip regeneration has also been shown to be nerve-dependent. This Miller lab paper described nerve-derived precursor cells from the endoneurium that become part of regenerating bone and dermis.

Dedifferentiated Schwann cell precursors required for regeneration



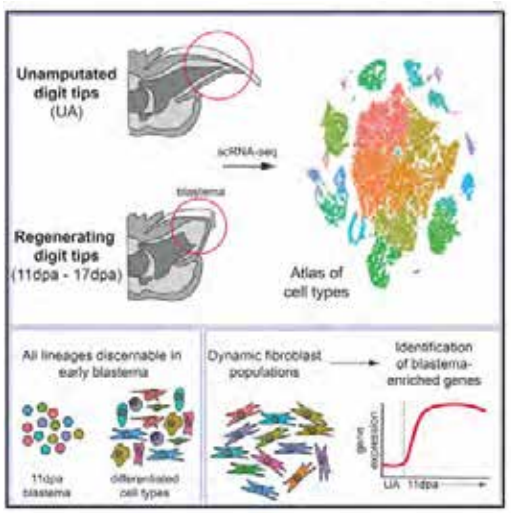
- Schwann cell precursors (SCPs) enter the regenerating digit tip
- Ablation of SCPs by inducible deletion or denervation inhibits regeneration
- SCPs secrete growth factors to promote mesenchymal cell proliferation
- Oncostatin M and PDGF-AA can rescue regeneration in a denervated digit tip

Johnston et al., 2016, Cell Stem Cell 19, 433–448

Miller's lab has also shown that Schwann cell precursors from nerves in the stump migrate to the blastema and secrete PDGF-AA and Oncostatin M, which promote proliferation of other cells in the blastema.

Denervate the digit or remove SCPs, regeneration fails; add the factors they secrete, regeneration is rescued.

Cell types in blastema:



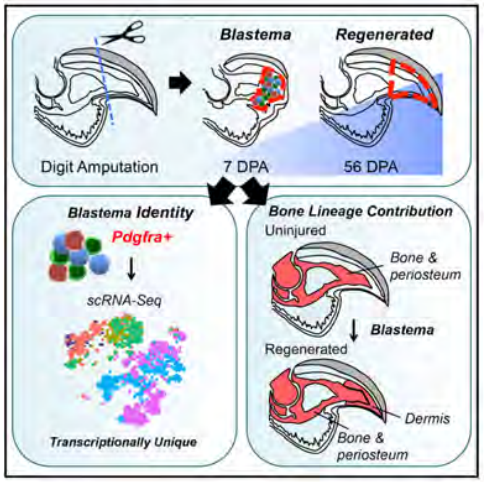
Highlights

- Unbiased atlas of cell types throughout mouse digit tip regeneration
- The early blastema contains all broad cell lineages found in the unamputated digit tip
- Fibroblasts are the most abundant and heterogeneous population
- Dynamics of distinct fibroblast populations reveal regeneration-specific markers

Johnson et al., 2020, Developmental Cell 52, 525–540

Using single cell RNA seq, Jessica Lehoczky's group has shown that all broad cell lineages present in the unamputated digit are represented in the blastema, including Schwann cells, macrophages, neutrophils, endothelial cells, osteoblasts, fibroblasts, T cells, monocytes, pre-osteoclasts, vascular smooth muscle cells, and lymphatic endothelium.

“Blastema-ness” determined by the environment

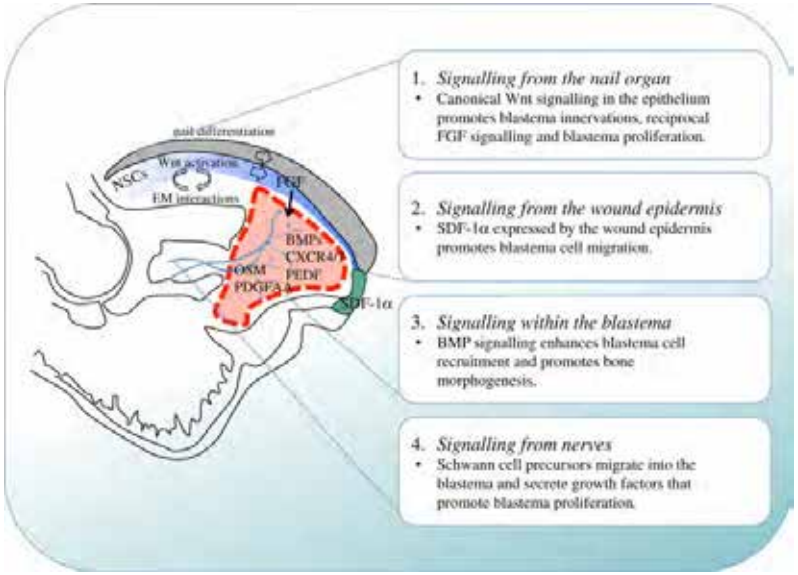


- *Pdgfra*-expressing mesenchymal cells from uninjured digits establish the blastema
- Adult digit tip regeneration is distinct from embryonic digit development
- The blastema state is environmentally determined
- The regenerative environment enables mesenchymal lineage plasticity

Storer et al., 2020, Developmental Cell 52, 509–524

How do these precursor cells change in the blastema? Miller’s group has shown that mesenchymal precursor cells of various backgrounds transition to a unique blastema transcriptional state—somewhere between developmental and adult states—upon relocation to the blastema. Something about that environment allows the cells to proceed with regeneration instead of simply forming scar tissue.

Summary of cellular and molecular signalling:



Storer MA, Miller FD. 2020 Cellular and molecular mechanisms that regulate mammalian digit tip regeneration. Open Biol. 10: 200194.

Like what? Miller's recent excellent review summarizes most of what's known about molecular signaling events in mouse digit tip regeneration.

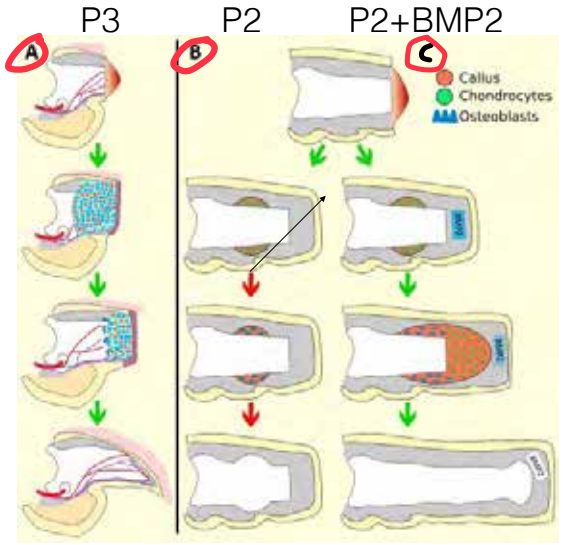
-It could be Wnt signaling—the nailfield (in blue) includes Nailbed SCs whose Wnt signaling drives periosteal cells to proliferate.

-It could be the wound epidermis (in green); it expresses SDF-1a, which interacts with receptors in the blastema to attract more progenitors.

-It could be BMP signalling in the blastema, shown here in red; BMPs recruit more progenitors and drive new bone formation.

-Lastly there are the Schwann cell precursors from injured nerves which migrate into the blastema and secrete factors promoting blastema proliferation.

‘Forcing’ regeneration: BMP2 rescues P2 amp



Dolan, Dawson, Muneoka 2018

So that’s what we know about how the unperturbed process works.

What if we manipulate the system?

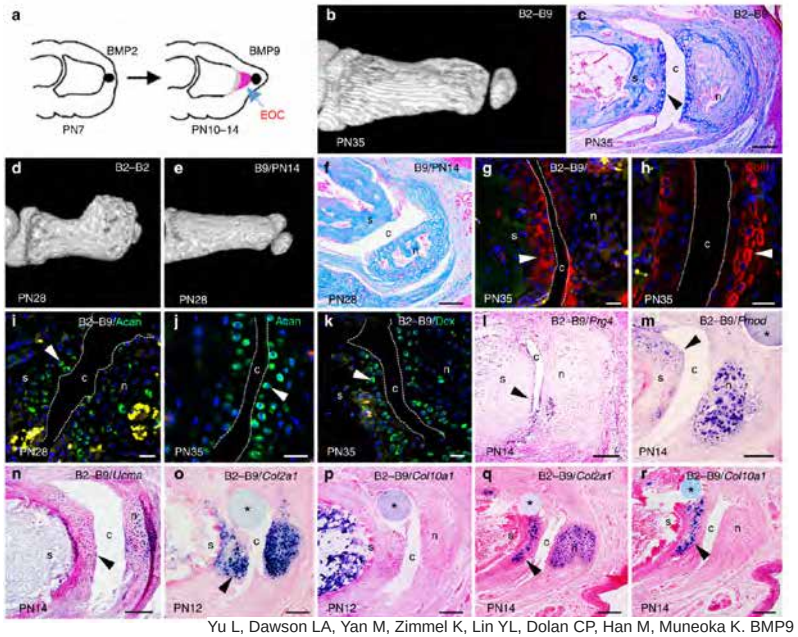
Knowing what we do now, can we make something regenerate where it usually would not?

The answer seems to be Yes. Ken Muneoka’s group has shown that amputations through P2–the middle phalanx—of the mouse digit, which usually results in a shortened stump, will regenerate the missing length of the bone if stimulated by BMP-2.

Then what? Does it make a joint and the rest of the digit? No.

Forcing a synovial digit joint:

BMP2>9 regenerate cartilage-lined articular space



Yu L, Dawson LA, Yan M, Zimmel K, Lin YL, Dolan CP, Han M, Muneoka K. BMP9 stimulates joint regeneration at digit amputation wounds in mice. Nat Commun. 2019 Feb 5;10(1)

Or maybe yes. Partially. The same group reported last year in Nature Communications that sequencing delivery of BMP2 followed by BMP9 leads to formation of a cartilage-lined synovial joint.

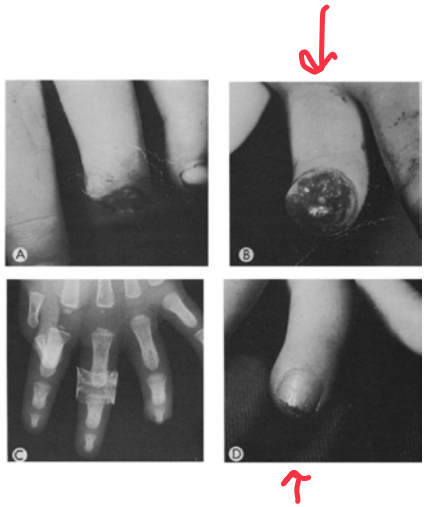
P3? They’re working on it.

So to summarize the mouse work to date:

The Key point: mammalian regeneration failure seems most likely to be due to a defective (“non-permissive”) wound environment, rather than any defect in the cells. Mammalian cells appear capable of participating in regeneration, if given the right signals and surroundings, even at locations that don’t ordinarily regenerate.

Graphic image warning

What about us?



Trapped Fingers and Amputated Finger Tips in Children

By Cynthia M. Illingworth

IN THE ACCIDENT AND EMERGENCY Department of the Children's Hospital, Sheffield, we see each year between 300 and 350 trapped fingers. The management of this injury has been modified with experience. We now know that spontaneous regeneration and excellent cosmetic and functional results can be obtained in guillotine amputations of finger tips in young children.

REVIEW OF THE LITERATURE

Many experiments have been made on animals in an attempt to produce regrowth of an amputated part. In the larval stage a salamander can regenerate a forelimb in 30–40 days, but the adult takes longer and the regenerated limb is short. In froglets the degree of regeneration depended on the stage of development—the younger the froglet the greater was the regeneration.[1] Regeneration was greater in the distal parts. Opossums were used as experimental animals[2] because they are in an early stage of development at birth. Regeneration of the limb occurred if they implanted nervous tissues into the proximal part of the limb before amputation. Others[3,4] have shown that regeneration is dependent on the presence of a critical amount of nervous tissue in the amputation stump and that if this is reduced, as in the salamander by cutting the nerve proximally,

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

On to humans.

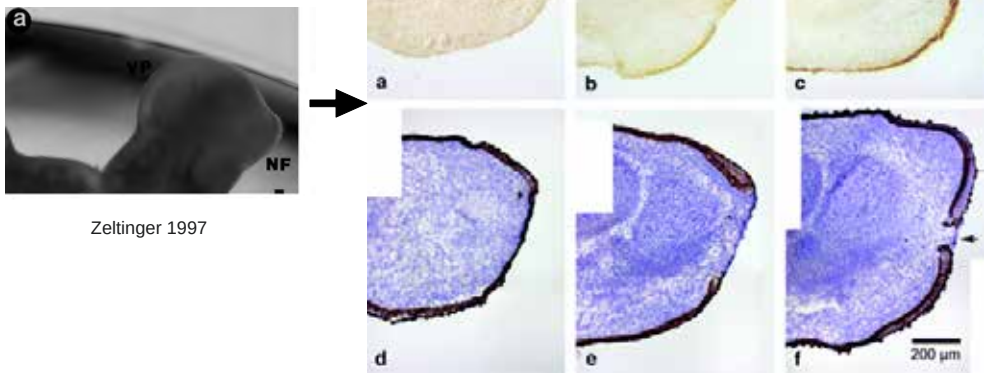
This is one of the earliest reports of digit tip regeneration in kids, in fact the paper that got me interested in the field. Upper right shows a fingertip injury and lower right shows it fully healed several weeks later with just dressing changes.

I saw this often in my own practice. Kids will regrow an amputated digit tip if it’s distal enough (through the nail unit) and if they’re young enough.

This seven year old girl lost her digit tip to her brother’s bicycle spokes.

The arrow points to the regrown tip.

Msx-1 expression in human fetal digits



Zeltinger 1997

Allan et al, Wound Rep Rgn 2006

How can we study this in any kind of controlled way?

This slide summarizes a decade of work on a suspension organ culture model of human fetal digit tip regeneration, with the main takeaway being that fetal digits do appear to have a population of mesenchymal precursor cells associated with the nailfield—as in the mouse—and that these cells help regrow amputated digit tips in vitro

Establishing and characterizing human P3 and P2 cell lines:
Do adult human digits retain regeneration-competent cells?

Tissue excised from beneath nail field, traumatically amputated nonreplantable adult human digits

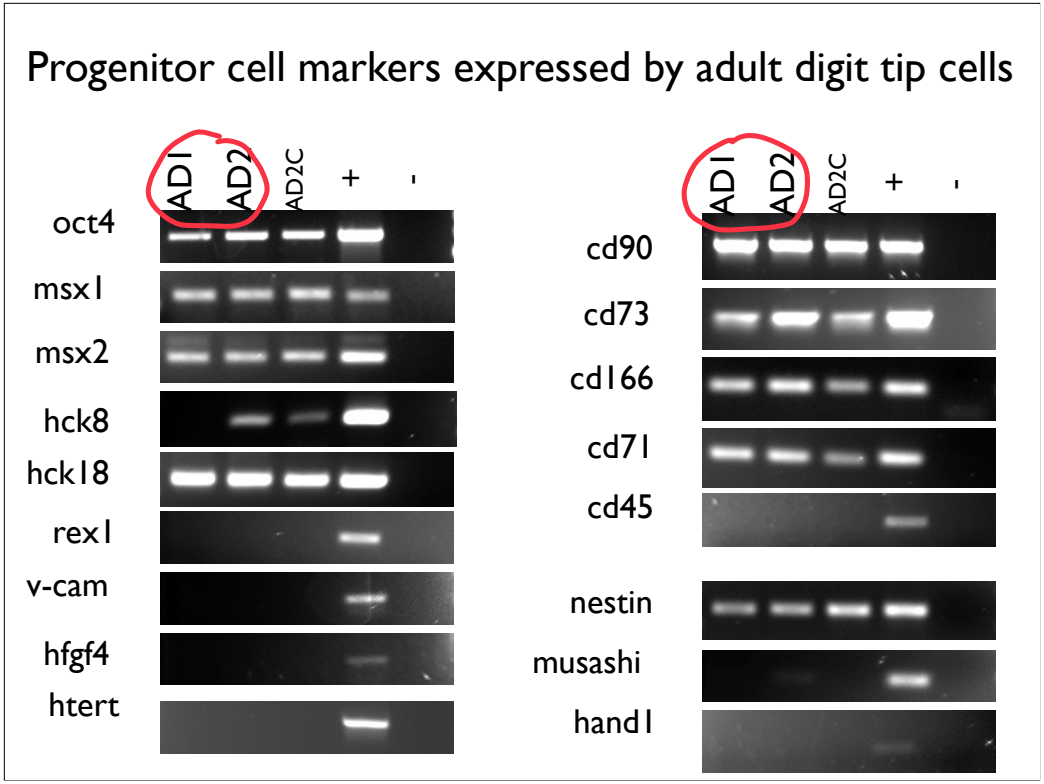
AD2 frozen, thawed and replated

~0.1% initial survival of cells

-can grow, divide and differentiate in culture

-can be frozen, thawed, and passaged in culture

To translate fetal digit observations to adult humans we collected fresh, traumatically amputated, nonreplantable adult human digits. We dissected the loose connective tissue mesenchyme from multiple sites and processed using a MSC-isolating protocol from Ken Muneoka's mouse work.

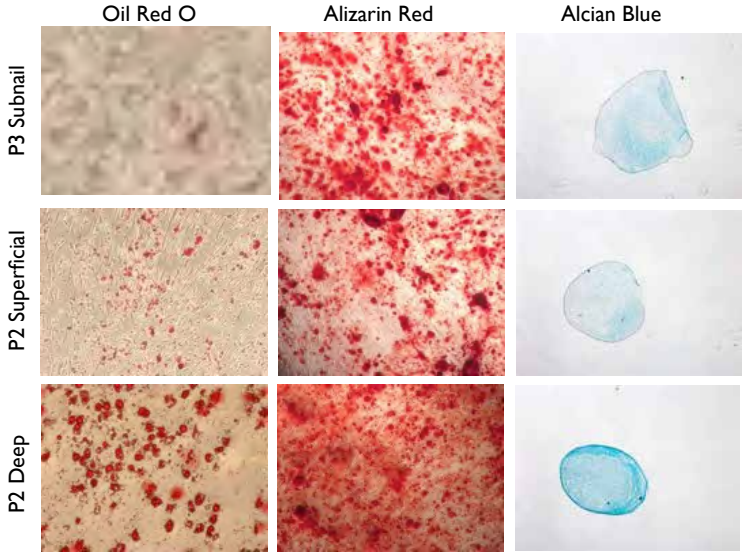


We were able to isolate a population of adult human digit-derived cells, and an outstanding postdoc in Randy Moon's lab at the time, Cristi Stoick Cooper joined us on our DARPA grant to look for progenitor markers using PCR and found several present—oct4, msx1&2, CD71, 73, 90 & 166 are all associated with precursor cells, multipotency, and/or neural stem cells.

And these adult digit tip cells were positive by IHC for several markers of neuronal precursors like S100 expressed by Schwann cell precursors and Nestin, for neural stem cells—reminiscent of the mouse.

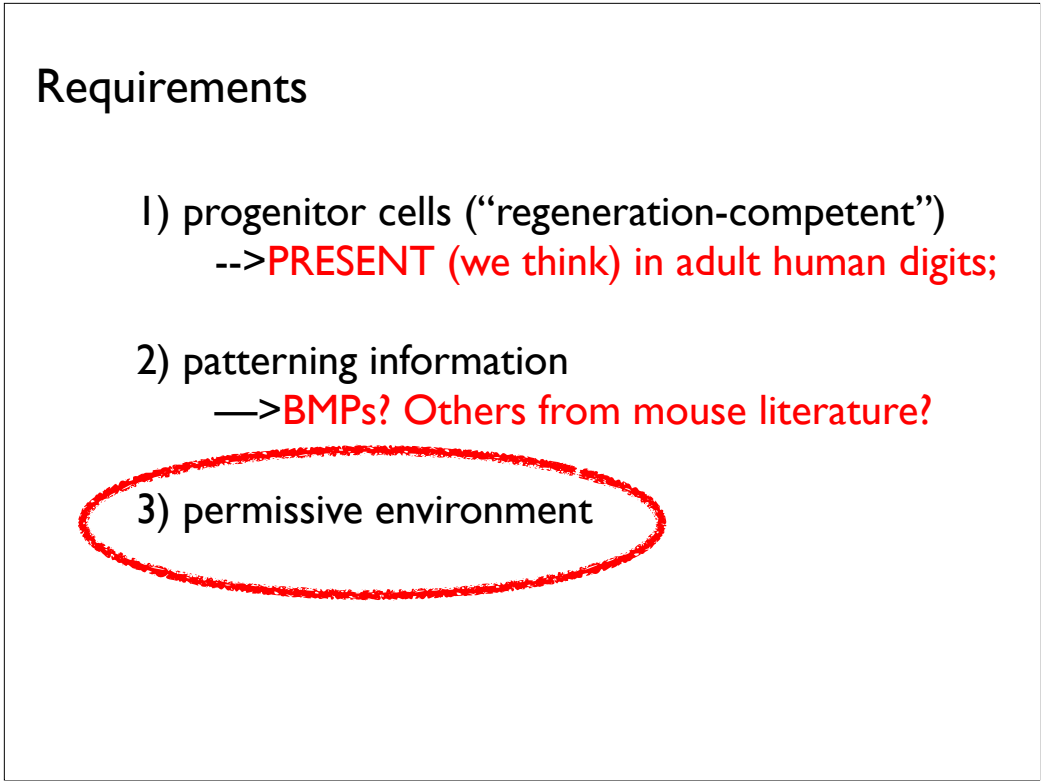
Differentiation potential:

-bone, cartilage, fat; regeneration-competent?



Cells isolated from 3 separate regions of an adult human digit have varying capacities for differentiating into fat, bone and cartilage.

To further ask about regenerative capacity or “stem-ness" we directed these digit tip cells down multiple lineages to produce fat, bone and cartilage--suggesting a multipotent precursor cell population persists in adult human digit tips.



So if we have regeneration-competent cells in adult human digits, that's one barrier overcome.

Now let’s look at the permissive environment.

Graphic image warning

Permissive environment, mouse digit tip

-protect regenerating part

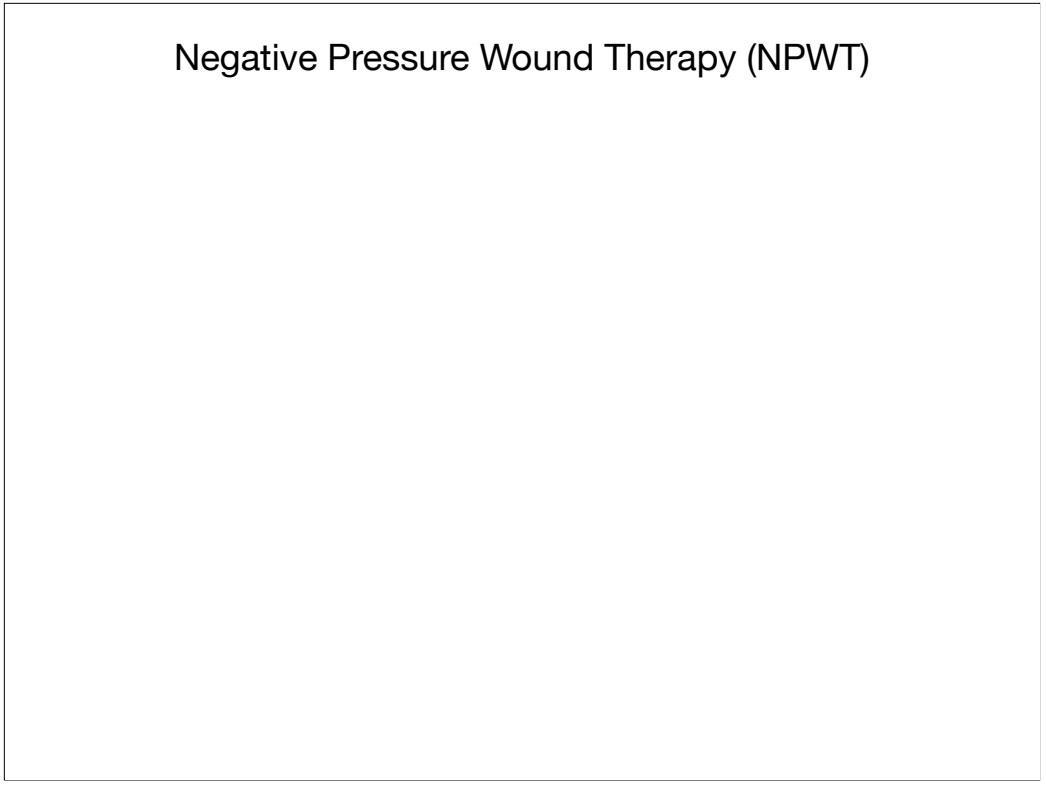
-provide mechanical signals

-allow for delivery of cells, factors, etc.

-Hechavarria et al, Med Eng Phys 2010

Here's one approach. Hechiavarra et al devised this "Biodome" for mouse digit tip amps. It allows for observation of the wound, addition of factors, mechanical and electrical stimulation, etc.

Negative Pressure Wound Therapy (NPWT)



One mechanical stimulus we use in other wound types is a vacuum dressing, or negative pressure wound therapy.

Negative Pressure Wound Therapy (NPWT)

Our lawnmower injury…

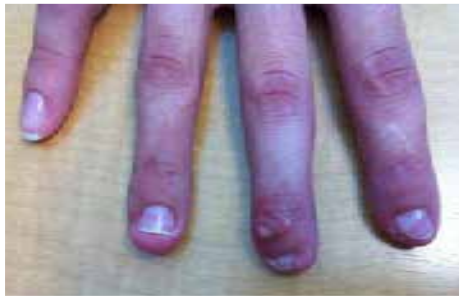
Negative Pressure Wound Therapy (NPWT)

Into a sterile foam vacuum sponge...

Negative Pressure Wound Therapy (NPWT)

Sealed airtight with a tube connected to suction…

Negative Pressure Wound Therapy (NPWT)



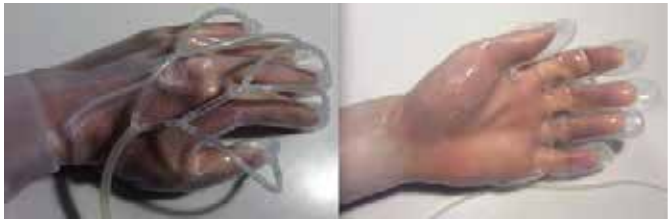
And when healed a result the patient was happy with.

We have had a collaboration for several years now with the University of Texas at Arlington Research Institute (UTARI) to tweak that mouse Biodome concept for human application, to address some of the areas where the dressing shown falls short—

we'd like to be able to see the wound, to leave the dressing on for longer than a week at a time, to get rid of the painful sponge removal, to deliver growth factors or other agents, to manipulate pressure, maybe culture cells in situ at the wound site...many possibilities.

Here’s a first effort, a Biodigit;

Bio-Glove



ReHeal Glove US Patent #10,004,884

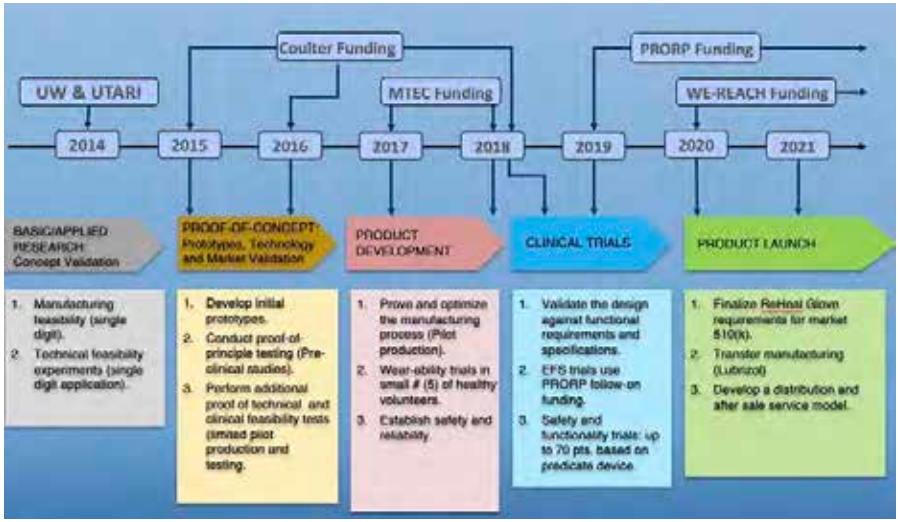
- Medical grade silicone: transparent for wound monitoring
- Self sealing strap: easy to apply and seal
- Allows full range of motion
- Non adherent; easy to remove

But we moved quickly to a glove format to address a wider range of injuries.

This allows easy application and removal, visibility for wound assessment, and full range of motion to prevent stiffness.

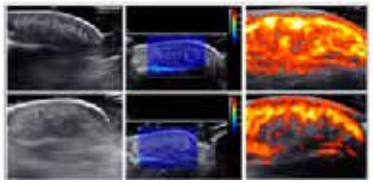
Here’s an earlier version in action (for demo video please see https://orthop.washington.edu/research/ourlabs/human-digit-regeneration-lab.html)

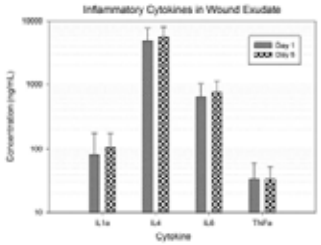
ReHeal Development Timeline



Back to the lab

Three recent human studies—observational, not interventional...



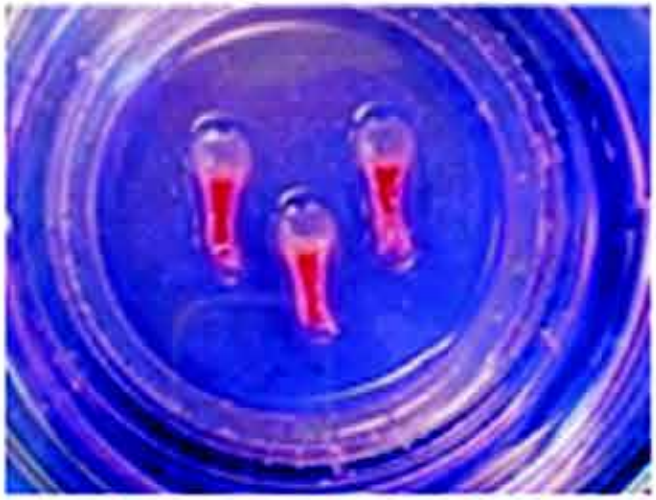


Schultz J et al. Conservative treatment of fingertip injuries in children - first experiences with a novel silicone finger cap that enables woundfluid analysis. GMS Interdiscip Plast Reconstr Surg DGPW. 2018 Oct 19;7

Jafari P et al. First Insights into Human Fingertip Regeneration by Echo-Doppler Imaging and Wound Microenvironment Assessment. Int J Mol Sci. 2017 May 13;18(5):1054

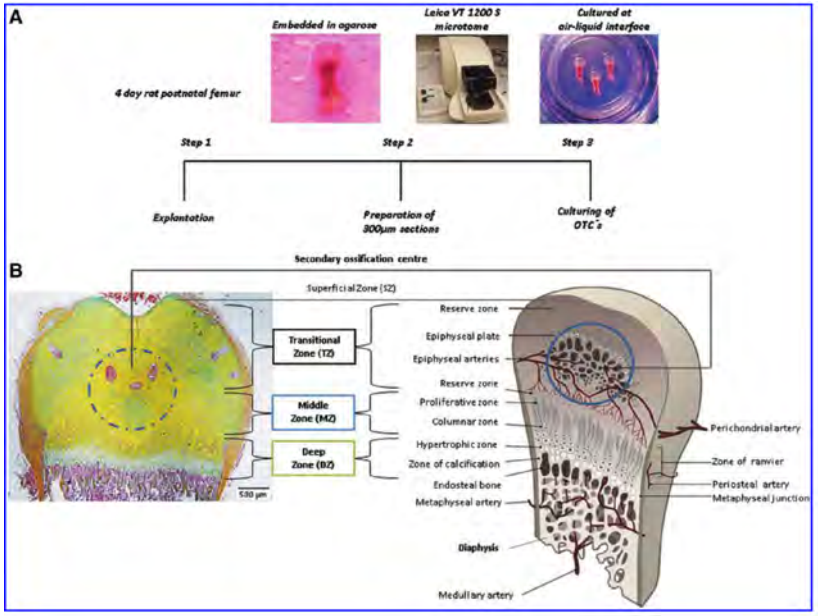
Kisch et al. Collection of Wound Exudate From Human Digit Tip Amputations Does Not Impair Regenerative Healing: A Randomized Trial. Medicine (Baltimore). 2015 Oct;94(41)

Need perturbable system/testbed: "Digit tip on a chip"



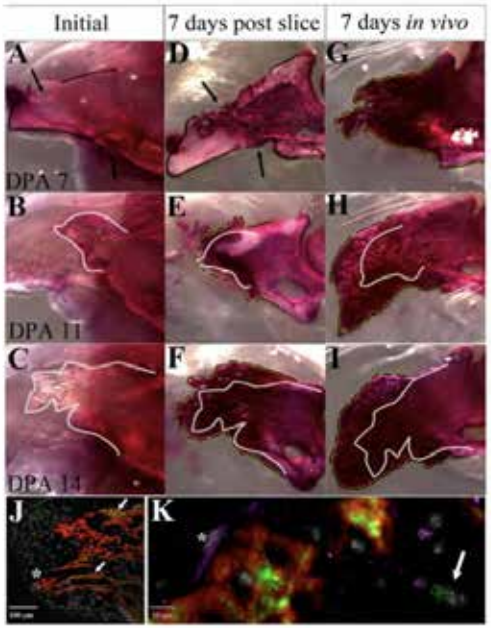
Lab Chip, 2020,**20**, 274-284

Slice culture



Srinivasaiah S, et al. A 300 µm Organotypic Bone Slice Culture Model for Temporal Investigation of Endochondral Osteogenesis. Tissue Eng Part C Methods. 2019 Apr;25(4):197-212.

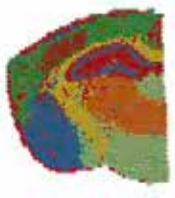
Mouse digits: regeneration in slice culture

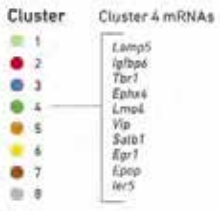


Sammarco et al, 2014

Near-term goals

- Establish baseline spatial genomics (Visium?)
- Develop survival cx conditions
- Test pathways from mouse work: Enhance P3 regeneration in vitro?
- P2, joints later





Longer-term goals: translate to Glove v. 2.0: Bench-to bedside vision for the future

'Permissive environment': deliver cells, factors; resorbable scaffolding as part of glove? Electricity? O2? Negative pressure? Positive pressure? Other?

-incorporate bone graft, cartilage scaffolds, decellularized cadaver parts

-allow for use of 3D bioprinted replacement parts (osseo)integrated into residual limb/hand bones

-allow for delivery of cells, factors

-stimulate regeneration & protect regenerating digits

-directed by basic science observations

Thanks!

ReHeal Glove team:
Brandon Bowman
Lynn Rose
Karen Adams
Muthu Wijesundara (UTARI)
Aida Nasirian (UTARI)
Alexandra Lindsey (UTARI)

UW Institute for Stem Cells and Regen. Med.
Moon Lab
Cristi Stoick-Cooper
Brent Fall
Andy Zhou
Ursula Holder
Justin Lee
Ashley Jaeger

UW Dermatology Research Center:
Rima Koulikovskas
Alicia Gutierrez
Jenny James
Zudtky Wisecarver
Phil Fleckman
Bob Underwood
Marcia Usui
Abby Navratil
Kathleen Berfield
Zerlina Wu
John Olerud

Kwon Lab!

The following funding sources are gratefully acknowledged:
WE-REACH
DoD CDMRP
Med. Tech. Enterprise Consortium (MTEC)
UW COULTER FUND
DARPA
Army Research Office (ARO)
UW Engineered Biomaterials
UW Dermatology Research Center

Thanks to the team members who did the work, and thanks to DARPA and ARO for their very necessary and very welcome support.