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.
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?
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 Fore toes

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.


Maybe we can learn from another mammal, the mouse.

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

Regeneration = multi-stage process

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.
This panel shows the process in stepwise fashion.
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.
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.
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.
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.
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.
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.
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.
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?

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.
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?

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.
Requirements

1) progenitor cells ("regeneration-competent")
   -->PRESENT (we think) in adult human digits;

2) patterning information
   -->BMPs? Others from mouse literature?

3) permissive environment

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.

Here's one approach. Hechavarra 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.
One mechanical stimulus we use in other wound types is a vacuum dressing, or negative pressure wound therapy.
Our lawnmower injury...
Negative Pressure Wound Therapy (NPWT)

Into a sterile foam vacuum sponge...
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

- 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

2014
UW & UTAR

2015

2016
Basic/Applied Research: Concept Validation

2017

2018

2019

2020

2021

Proof-of-Concept: Prototypes, Technology and Market Validation

Product Development

Clinical Trials

Product Launch

1. Manufacturing feasibility (sample digit).
2. Technical feasibility experiments (single digit application).
3. Perform additional proof of technical and clinical feasibility tests, pre-launch production and testing.

1. Develop initial prototypes.
2. Conduct proof-of-principle testing (preclinical studies).
3. Perform additional proof of technical and clinical feasibility tests, pre-launch production and testing.

1. Procurement and optimize the manufacturing process (Pilot production).
2. Wearability trials in small # of healthy volunteers.
3. Establish safety and functionality.

1. Validate the design against functional requirements and specifications.
2. EPS trials use "PROOF" before launching.
3. Safety and functionality trials up to 70 pts. based on previous device.

1. Finalize HCT/P (slice requirements for market 510(k).
2. Transfer manufacturing (UK/US/CA)
3. Develop a distribution and after-sale service model.
Three recent human studies—observational, not interventional...


Need perturbable system/testbed: “Digit tip on a chip”
Slice culture

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


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