

# Biomedical Lessons from Spiders and Axolotl

**Kerstin  
Reimers  
Labor**  
für Regenerationsbiologie

**MHH**

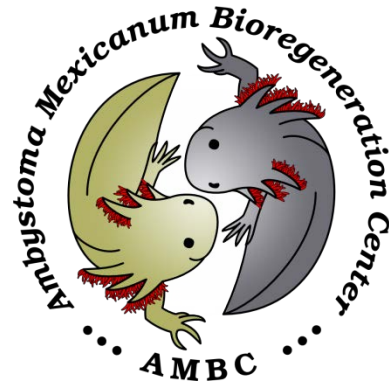
Hannover Medical School



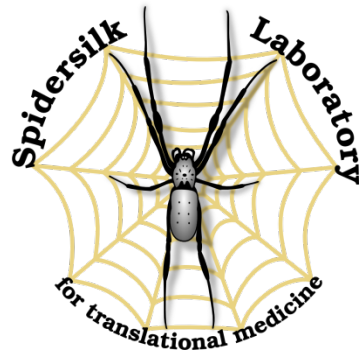
Prof. Dr. rer. nat  
Kerstin Reimers-Fadhlaoui

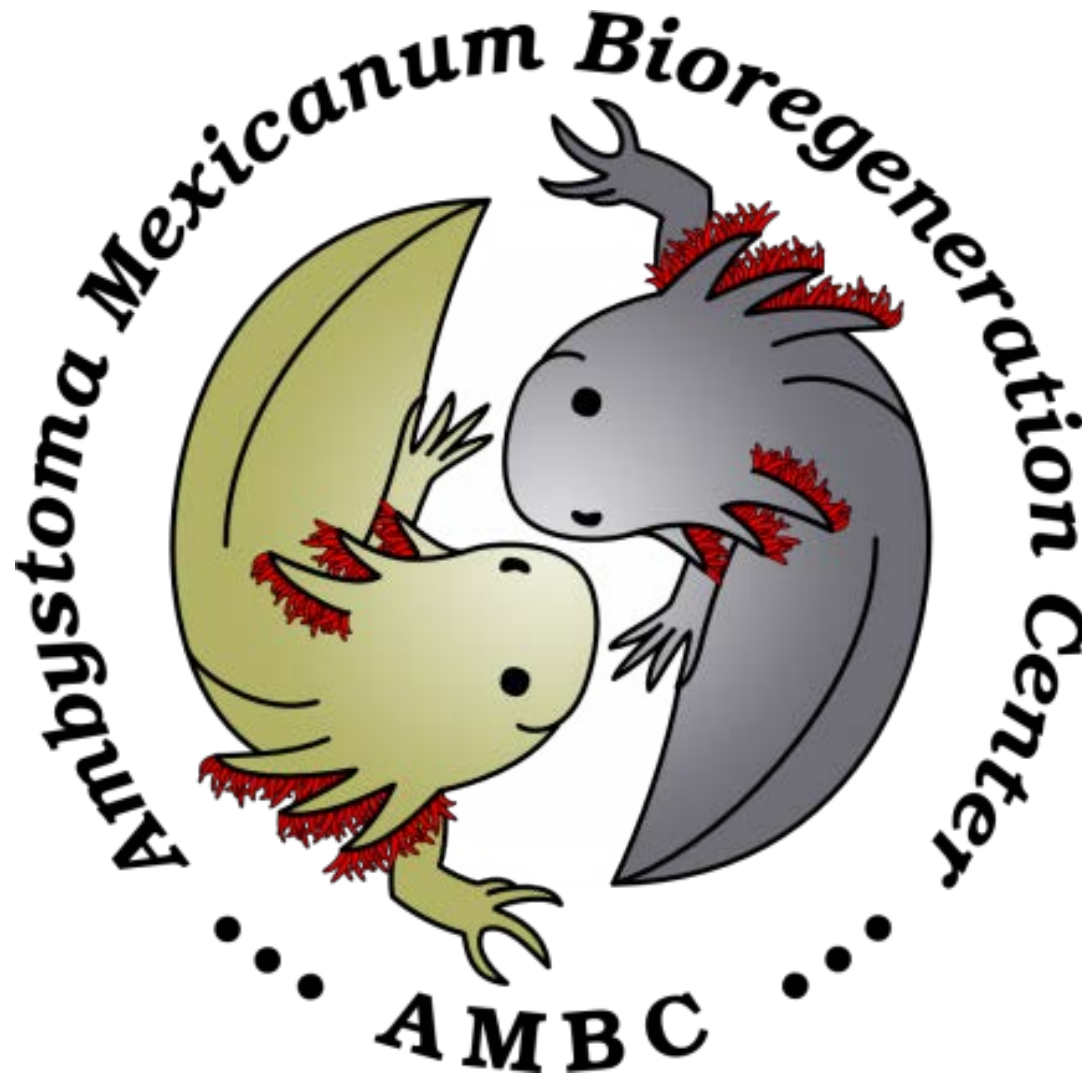
\* 16.01.1970  
† 23.12.2015

## Part I

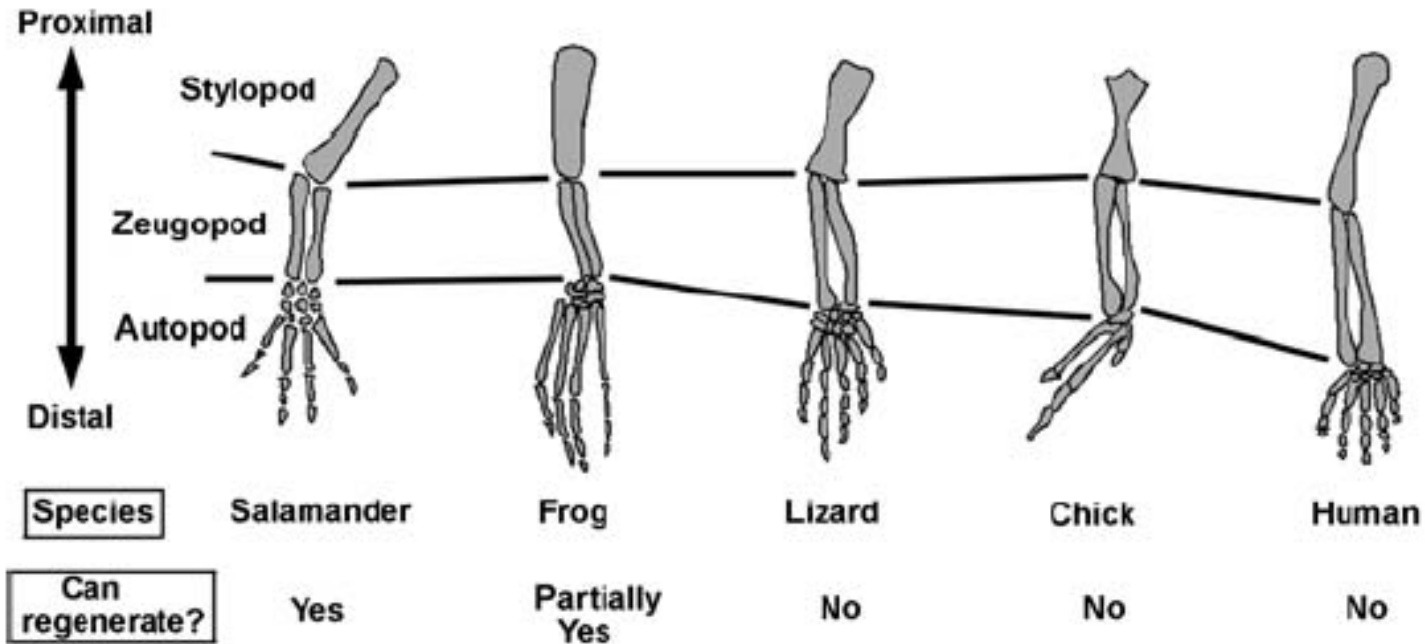


## Part II





# Regeneration capacities



(Yokoyama, 2008)

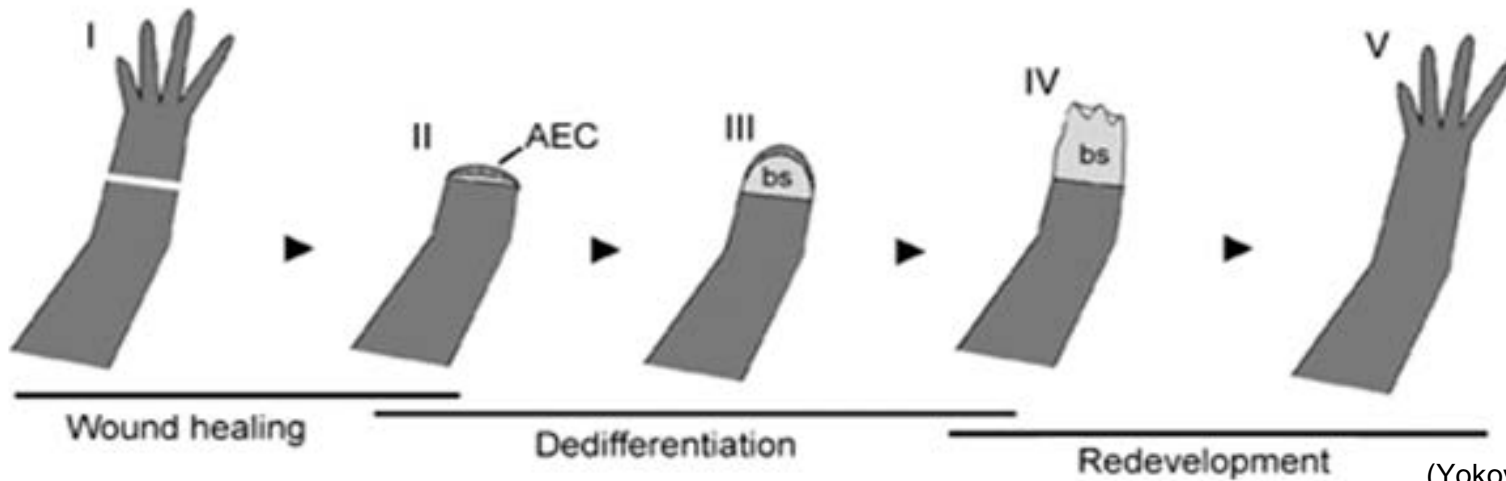
# Introducing the „supermodel of wound healing“

Kingdom	Animalia (animals)
Phylum	Vertebrata (vertebrates)
Superclass	Tetrapoda (quadrupedes)
Class	Amphibiae (amphibians)
Order	Caudata (caudates)
Suborder	Ambystomatoidea
Family	Ambystomatidae (Mole Salamanders)
Genus	Ambystoma
Species	<i>Ambystoma mexicanum</i> (Axolotl)



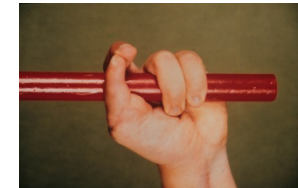
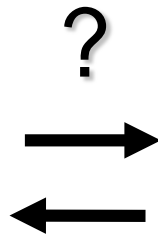
# Phases of limb regeneration

1. Wound healing
2. Dedifferentiation (apical endothelial cap)
3. Proliferation
4. Redifferentiation/pattern formation



(Yokoyama, 2008)

# Axolotl and human medicine?

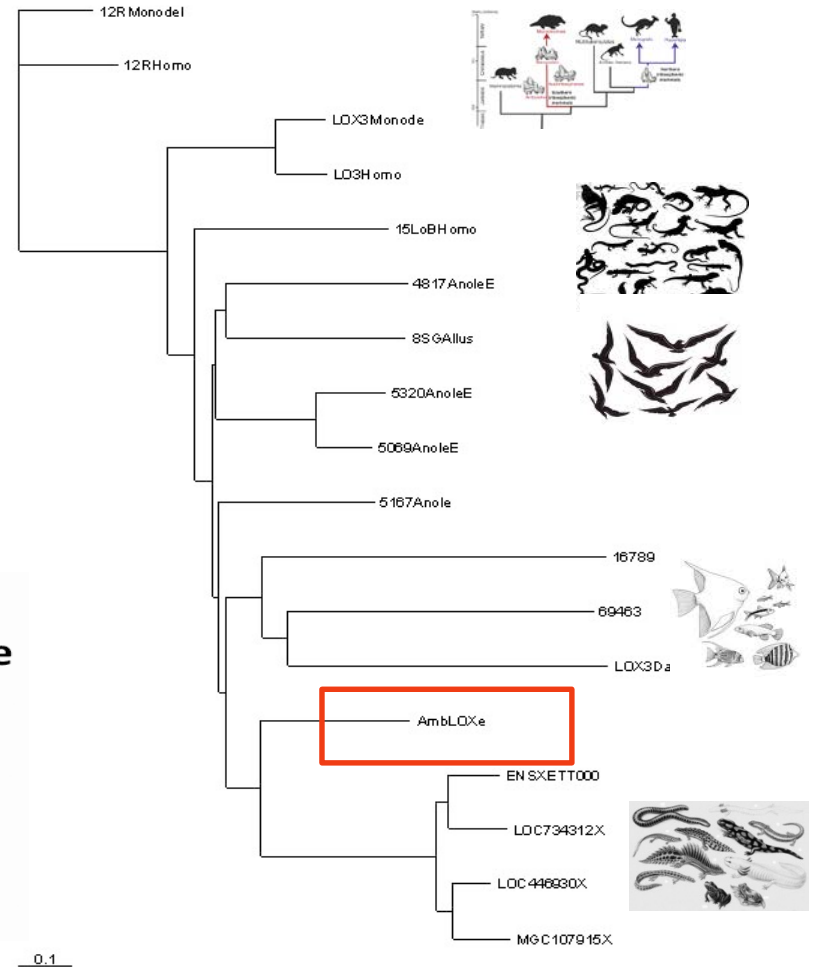
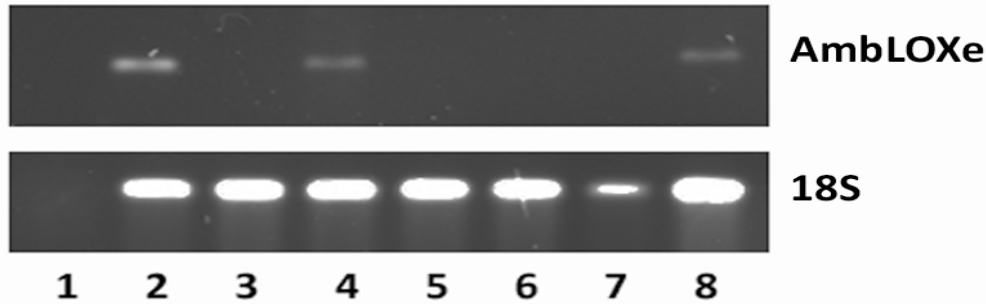


# AmbLOXe

→ lipoxygenase

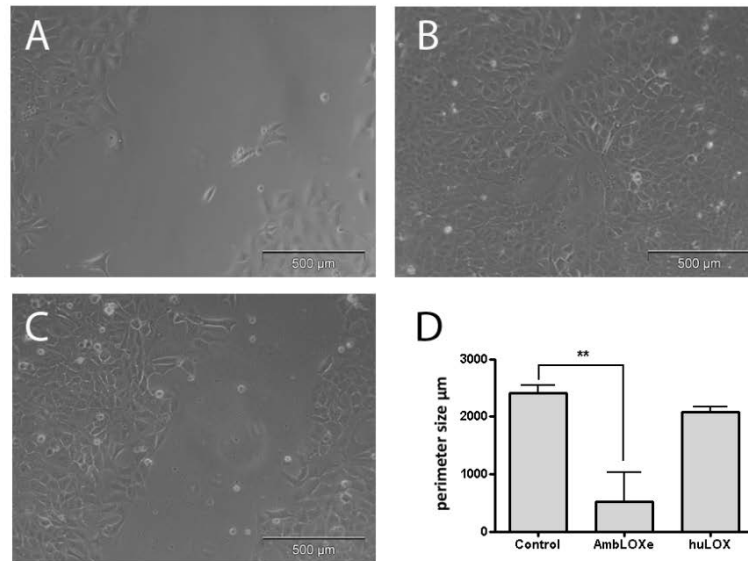
expression in blastema (2), skin (4),  
embryo (8)

no expression in foot (3), muscle (5),  
brain (6), bloodcells (7)



GenBank: EU814616, FJ213779 Reimers et al.

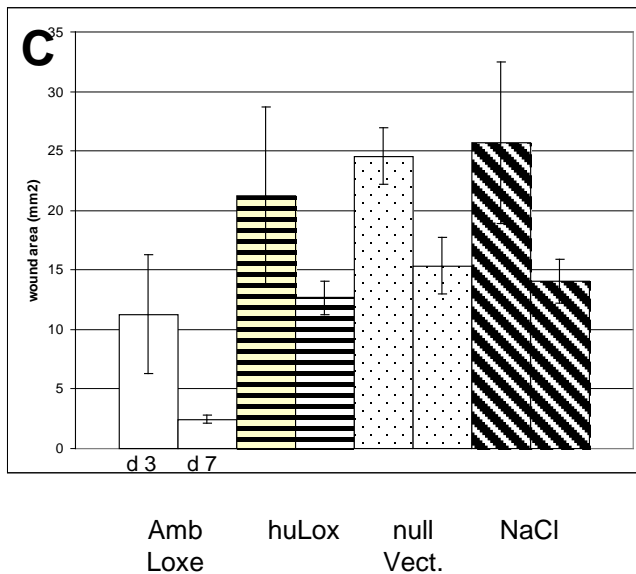
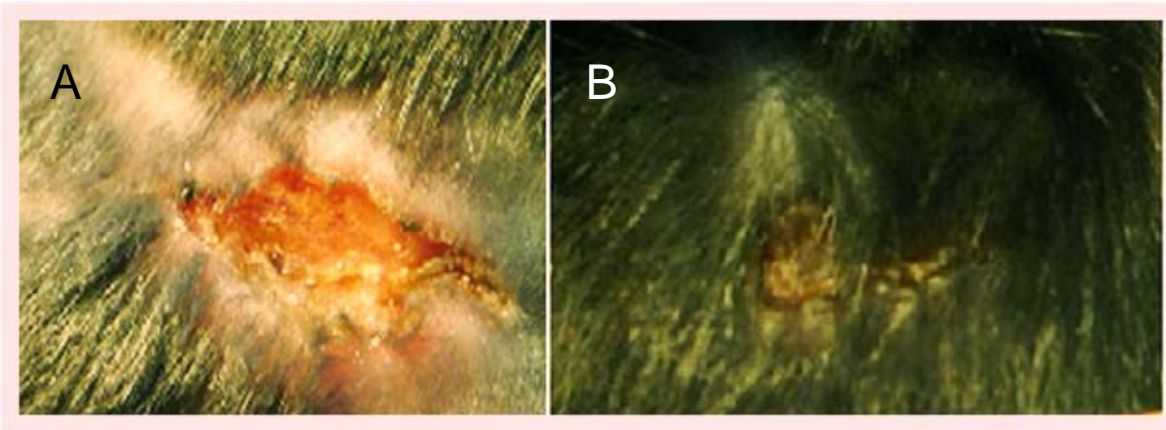
# AmbLOXe expression in human cells



A: no LOX  
C: huLOX

B: AmbLOXe  
D: wound area  
after 16h

# Wound treatment with AmbLOXe



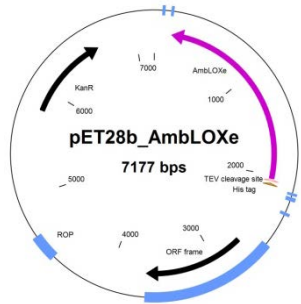
A: no LOX

B: AmbLOXe

C: wound area after 3 and 7 days

**Patent: Reimers: PCT/EP09007190.3**  
*„lipoxygenase and its use in wound healing”*

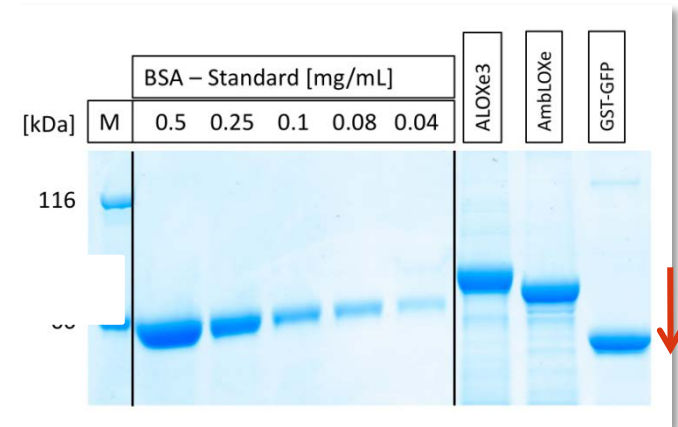
# AmbLOXe Synthesis in *E. coli*



Expression in *E. coli* BL21(DE3)  
pET28b\_His-TEV-AmbLOXe

His-Tag @ N-terminal  
TEV-protease site

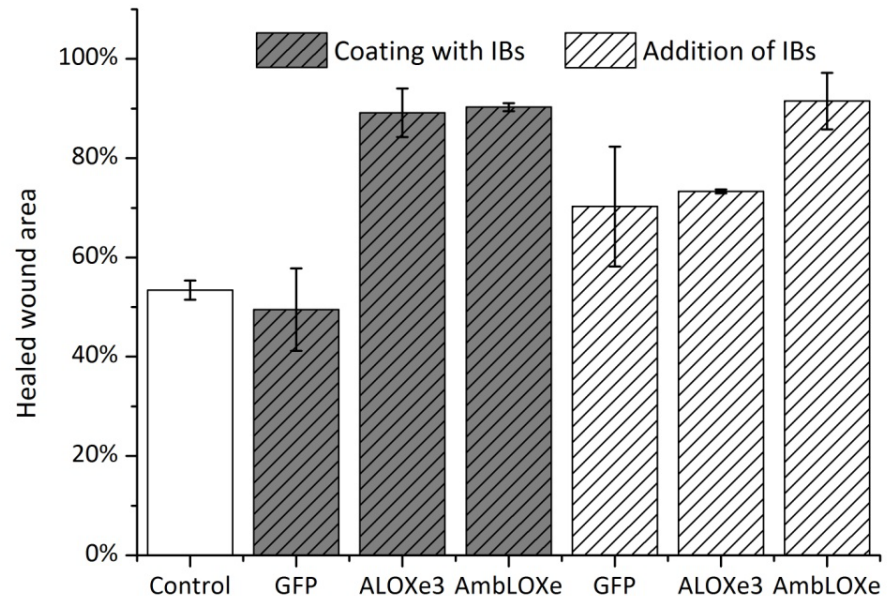
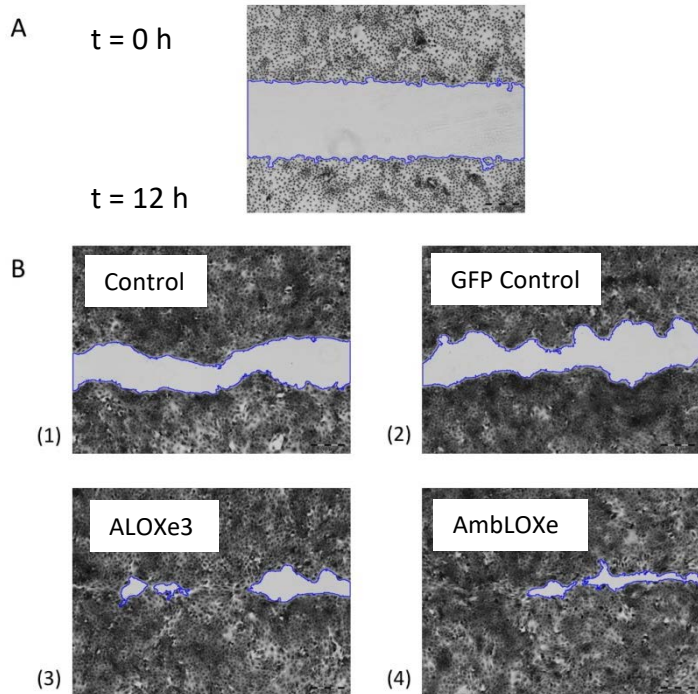
ALOXe3 and GFP as controls



DNA sequence verified

AmbLOXe produced & purified as inclusion bodies ✓

# Recombinant AmbLOXe



**Results of wound healing assay; Proportion of healed wound area (t = 12 h)**



Positive effect of AmbLOXe inclusion bodies on wound healing

# Some facts...

- natural habitat: lakes Xochimilco and Chalco in Mexico City  
→ **endemic species!**
- Xochimilco also known as “swimming gardens”  
→ chinampa agriculture, small islands surrounded by canals  
→ popular tourist destination
- water contamination by agriculture, tourism and untreated wastewater from Mexico City



→ **The Axolotl is listed as critically endangered!**

# Biomedical Research and Species Conservation???

species-appropriate keeping



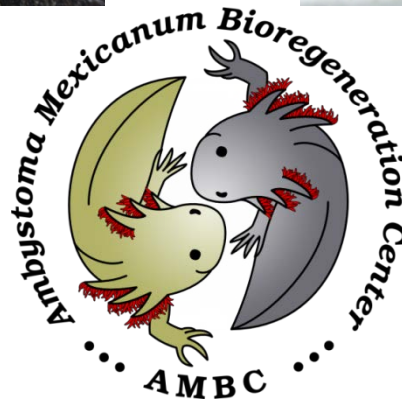
support for veterinarians

no trials with wild animals



advice of public authorities

successful breeding



education of pupils and students

development of in vitro models



press and public relations, lobby work



# What Axolotl Teach Us ...

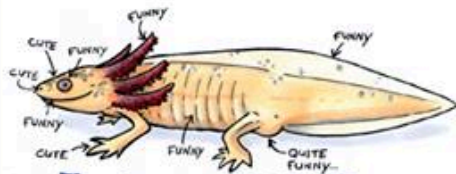
AmbLOXe – a key to regeneration?

- faster wound closure and reduced scarring when transferred in mammal in vitro and in vivo models
- regeneration of whole limbs and organs still remains a challenge
- molecular signaltransduction still unknown
- lipoxygenase type still unknown

Species protection and biomedical research are no opposites!

# WHY AXOLOTLS ARE AWESOME

BY MORRISON3000



**They look cute and funny**



**They come in different colors**



**They are curious**



**They have awesome external gills**



**They love to nibble your finger**



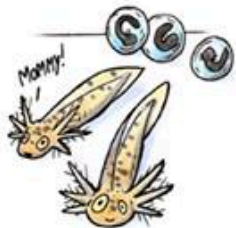
**They 'smile' and look good in pictures**



**They're not dangerous**



**They look funny when they yawn**



**They create cute babies very easily**



**They can regenerate bodyparts**



**They're relatively cheap**

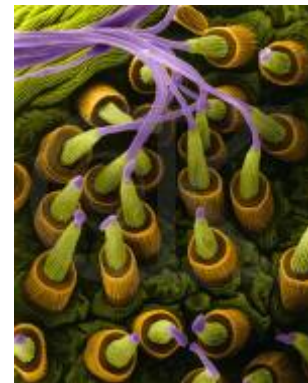
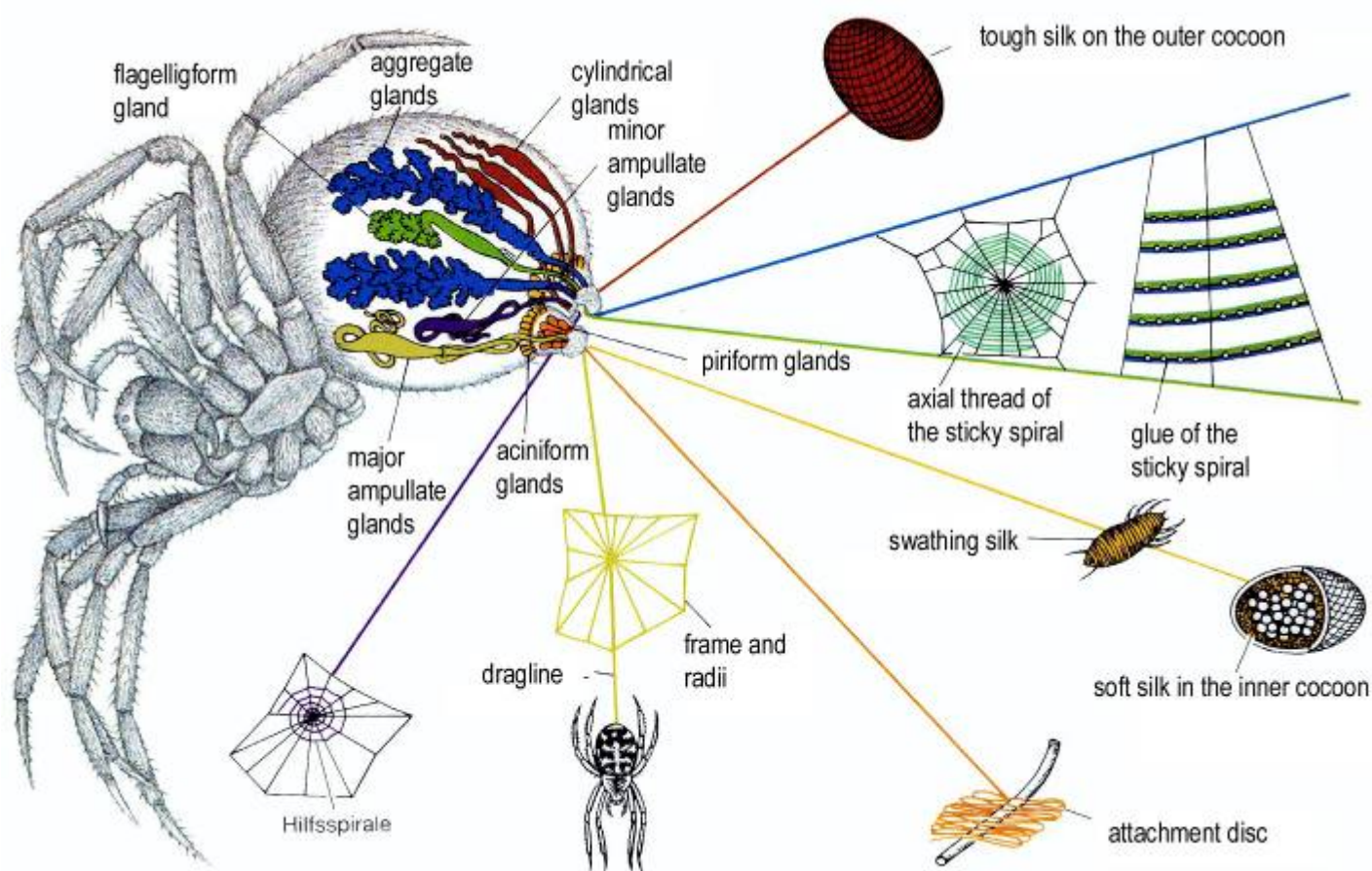


**They love to create 'axie stacks'**

 [facebook.com/morrison3000art](https://www.facebook.com/morrison3000art)



# Silk glands



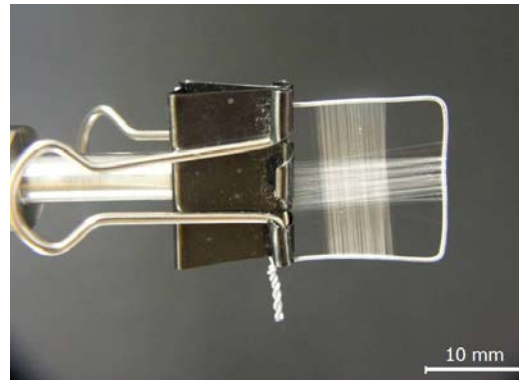
# Why spider silk?

- Tensile strength
- Elasticity
- Thermostability up to 250 °C
- Resistent against organic and anorganic solvents
- Excellent biocompatibility
- Good adhesion and proliferation of various celltypes on silk fibers

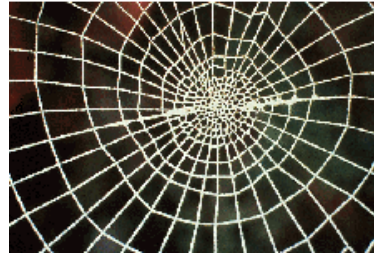


Prof. Shigeyoshi Osaki  
Nara Medical University  
Japan.

# Spider Silk Reeling



# Spider Silk for Biomedical Applications



Native spider silk

## Biomaterial

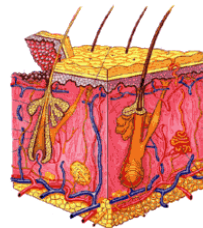
## Tissue Engineering



Sutures



 Nerve Regeneration



 Skin



Tendon

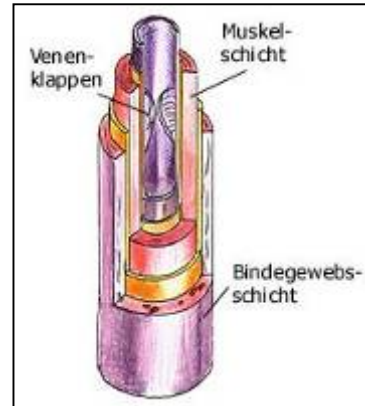
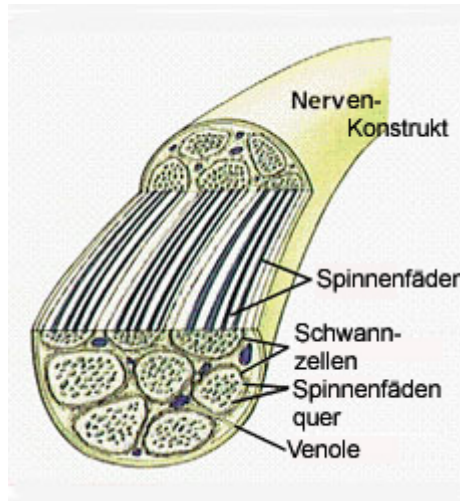


 Cartilage

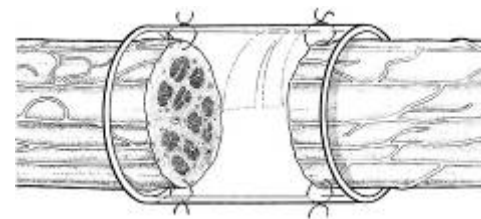


Bone

# Nerve Conduits



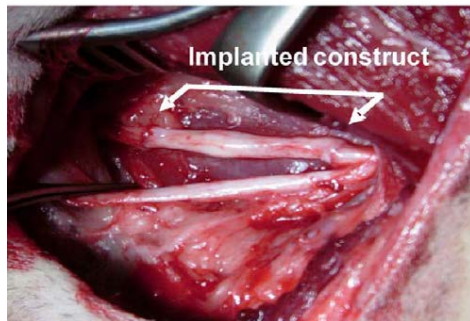
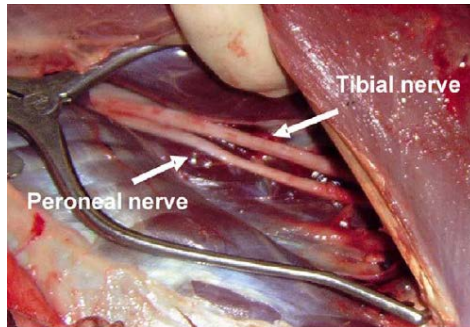
decellularized vein



Allmeling et al 2006, Allmeling et al 2008

# Nerve Conduits

Nerve regeneration of N. tibialis in sheep

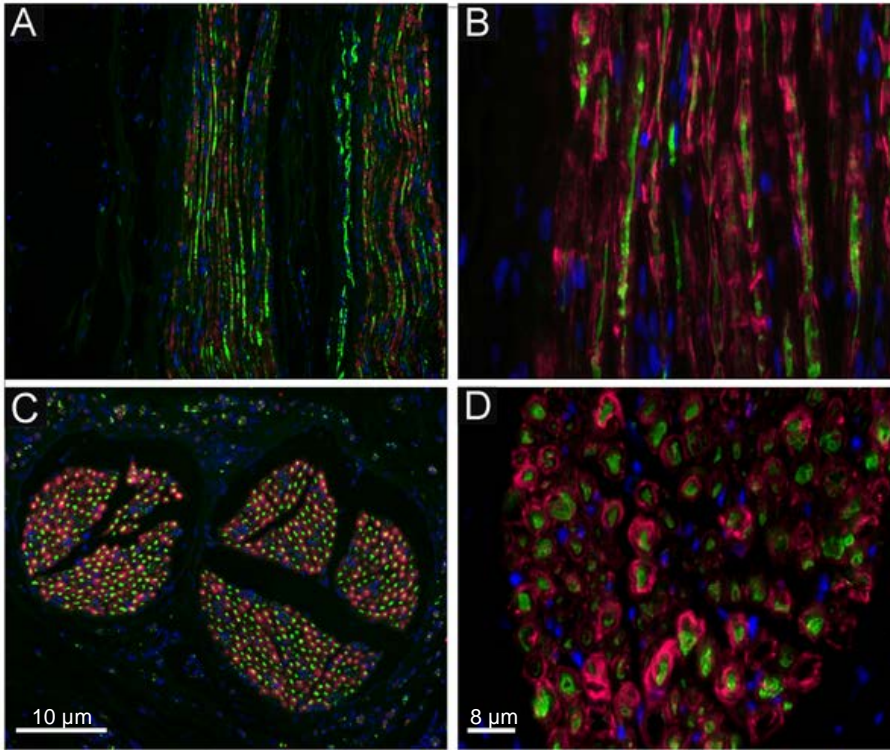


surgery situs  
length of defect: 6 cm

postoperative treatment

# Nerve Conduits

Nerve regeneration of N. tibialis in sheep



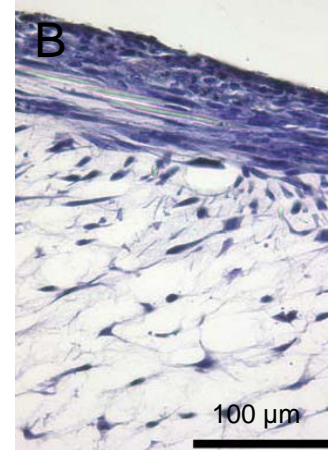
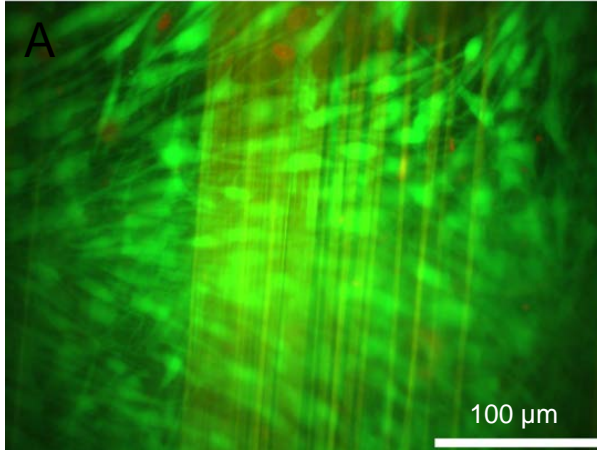
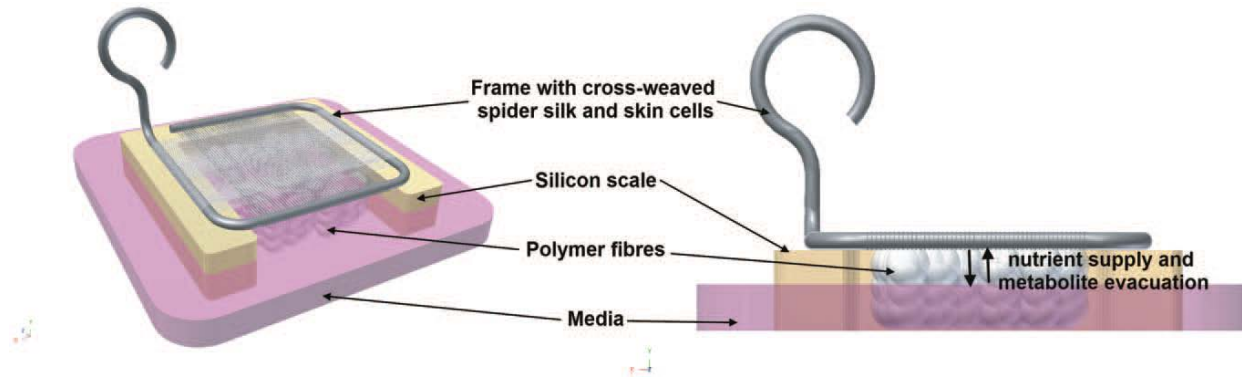
6 months after transplantation

green: neurofilament (regenerated axons)  
red: S100 (Schwann cells)  
blue: Nuclei

A&B: longitudinal sections  
C&D: cross sections

Radtke et al 2011

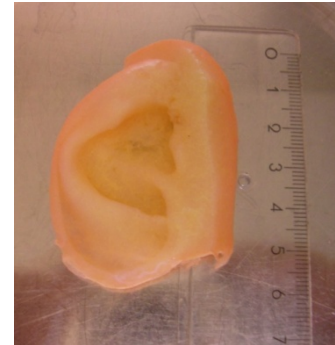
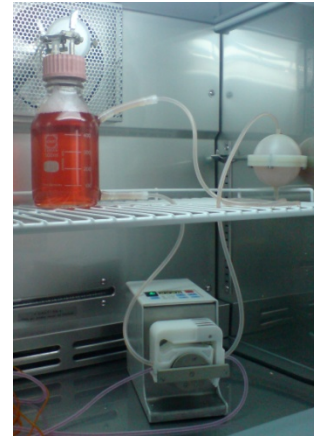
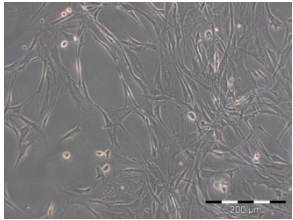
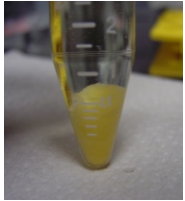
# Skin Tissue Engineering on Spider Silk



A: day 35 of cultivation  
green: vital cells  
red: dead cells  
B H&E staining

Wendt et al 2011

# Cartilage Tissue Engineering



# Availability...?



Godley and Peers 2012

# What Spiders Teach Us ...

## Spider silk

- is a multi-versatile biomaterial for tissue engineering
- can help to regenerate large peripheral nerve defects
- recombinant material is not identical to native product
- reeling is not harmful for the animals
- production in native habitats offers perspectives for local development



M<sub>H</sub>H

Hannover Medical School