



Nuclear Energy for National Projects

October 22-27, 2017

Dayrell Hotel & Convention Center
Belo Horizonte, MG, Brazil



The Use of Radiosterilized Fish Skin To Treat Burn Victims

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ACKNOWLEDGMENT



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SPONSORED BY



Assistance To Burned People Institute

Ceará



Brazilian Electrical Company
COELCE / ENEL

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SUPPORTING INSTITUTIONS



Ceará



Pernambuco



Nuclear and Energy Research Institute

São Paulo



Ceará



Laboratório de Imunopatologia Keizo Asami

Pernambuco



Pernambuco

Belief



Tilapia Fish Skin used for Handicraft.

The skin of Tilapia can be used to manufacture bags and shoes, but for the time it is being stocked. The idea is to make it become useful for handicraft production during off-season.

November/2011



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A MAJOR QUESTION CAME TO MY MIND

If the skin has enough resistance and delicacy to become a feminine accessory, why would it not have the same resistance and delicacy to replace temporarily human skin in burn treatment?



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WHY TILAPIA FISH - KNOWLEDGE

- ✓ It is highly resistant to catching diseases;
- ✓ Antimicrobial action – Defensin/Hepcidin/IL-1 β ?
- ✓ It has an early reproductive capability – plenty raw-material;
- ✓ Fresh water – aquatic environment allows management of water quality control and lower risk of disease transmission;



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**KNOWLEDGE: PHYSICIAN IN CHARGE OF THE SKIN BANK
HELD AT IMIP, IN RECIFE.**





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- ✓ CHLORHEXIDINE.
- ✓ GLYCEROL at high concentration.
 - Hyperosmolar environment (bacteria);
 - Hygroscopic action.
- ✓ Additional radiosterilization (virus).
 - Cobalt 60 gamma radiation.



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THEY MADE THE DIFFERENCE



MD. Edmar Maciel
General Coordinator



PhD. MD. Odorico Moraes
Scientific Coordinator



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PRECLINICAL PHASE

24 MONTHS

LABORATORIES

ANIMALS



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DRDC – UFC – 70 Team Members



Tilapia Fish
Skin
HE 200x

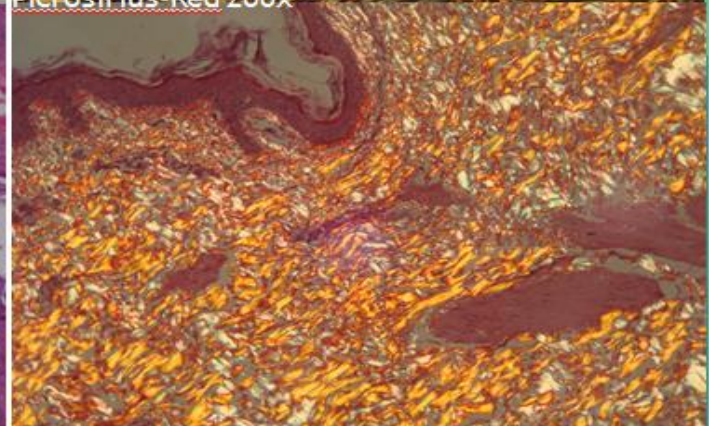
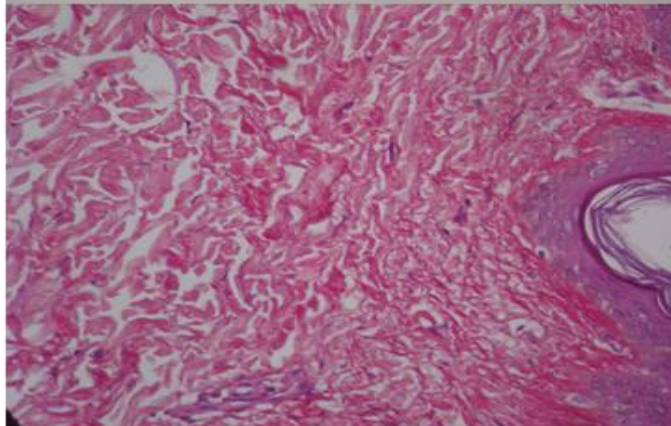
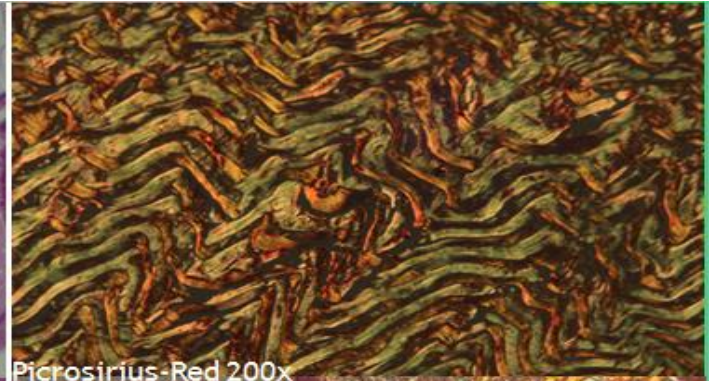
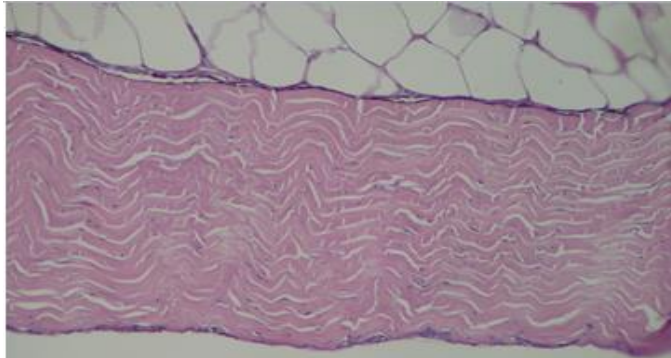
DOUBLE

Ratio Collagen Type I:III

Human: $0,65 \pm 0,06$

Tilapia: $1,12 \pm 0,15$

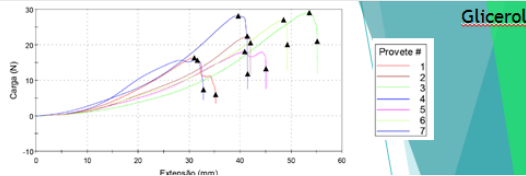
Human
Skin
HE 200x



Comparative Histological Study Between
Tilapia Fish Skin And Human Skin

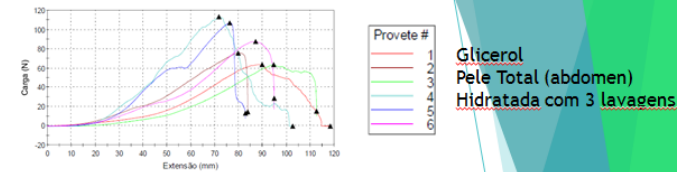
Skin Tension Study

Tilapia Skin



	Máx. Carga (N)	Deformação à tração em Máx. de Carga (%)	Carga em quebra (N)	Esforço à tração em quebra (N/mm ²)	Extensão à tração em quebra (cm)
1	16,428	30,975	6,082	0,704	3,52
2	22,506	41,342	20,615	2,386	4,20
3	29,080	53,517	21,066	2,847	5,51
4	15,763	31,567	7,478	1,011	3,28
5	18,189	40,850	13,363	1,407	4,50
6	27,074	48,467	20,120	3,183	4,92
7	28,184	39,583	11,930	1,326	4,14
Coef. de Variação	25,53	20,06	43,98	52,31	17,96
Máximo	29,080	53,517	21,066	3,183	5,51
Mínimo	15,763	30,975	6,082	0,704	3,28
Média	22,46	40,90	14,37	1,837	4,295
Desvio Padrão	5,735	8,2055	6,325	0,961	0,771

Human Skin



	Máx. Carga (N)	Deformação à tração em Máx. de Carga (%)	Carga em quebra (N)	Esforço à tração em quebra (N/mm ²)	Extensão à tração em quebra (cm)
1	63,730	89,775	-0,073	-0,009	11,83
2	75,578	79,025	14,786	1,760	8,30
3	63,609	86,717	15,512	1,633	10,47
4	113,373	71,725	-0,064	-0,006	10,26
5	107,046	76,225	13,440	1,292	8,28
6	87,661	87,075	28,787	3,129	9,48
Coef. de Variação	25,16541	8,74987	90,43851	91,58495	14,05588
Média	85,166	81,757	12,064	1,300	9,77
Desvio Padrão	21,43242	7,15363	10,91091	1,19052	1,37339

Extentionry Test: Human: 4.615 cm / Tilapia 4.442 cm

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2015/Jul., Aug., Sep. – Pg. 203 to 210



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MICROBIOTA STUDY OF 20 FISHES

- *Aeromonas hydrophila*
- *Aeromonas sóbria*
- *Aeromonas veronii*
- *Candida parapsilosis*
- *E. Coli*
- *Globicatella sanguinis*
- *Klebsiella pneumoniae*
- *Kocuria varians*
- *Pseudomonas aeruginosa*
- *Pseudomonas stutzeri*
- *Sphingomonas paucimobilis*
- *Streptococcus suis*
- *Streptococcus uberis*

< 100,000 CFU/g of tissue

Skin & Oral Cavity

PUBLISHED BY UNICHRISTUS JOURNAL



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PROCESSING TILAPIA FISH SKIN

From Castanhão Weir to DRDC

- Skin Removal;
- Washing Skin Under Running Water;
- Skin Inside Plastic Containers, In An Isothermal Box, is taken to Fortaleza.



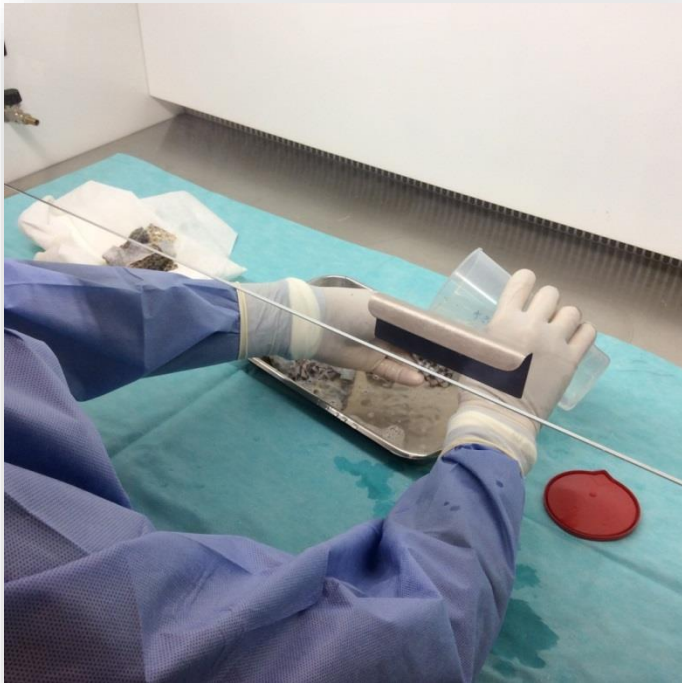
PROCESSING TILAPIA FISH SKIN - DRDC

- Cleansing Remaining Pieces of Muscles;
- Cutting Skin Out Into Pieces Of 10X5cm;
- C1 - Chlorhexidine at 2% Concentration for 1 hour;
- G2 - Glycerol at 75% Concentration + 25% Concentration Salt Solution, for 1 hour.



Laminar Flow

- G3 - Glycerol at 100% Concentration + Warm Water Bath at 15rpm, 37°C – for 3 hours;
- Storage – Double Sealed at 4°C, Sterile Double Plastic Envelopes.





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Irradiated Skin – Nuclear and Energy Research Institute (IPEN)





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Irradiated Skin – Nuclear and Energy Research Institute (IPEN)



Mônica Mathor – IPEN Researcher



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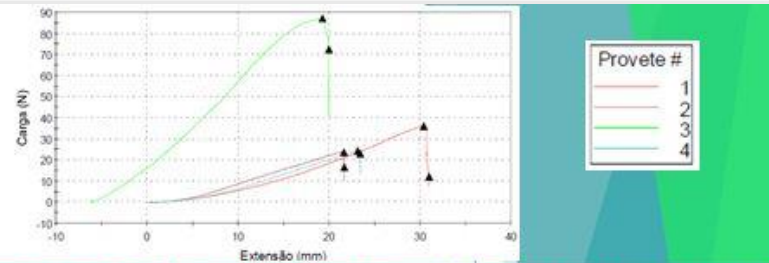
Irradiated Skin – Nuclear and Energy Research Institute (IPEN)



Delivery Box 4°C

Irradiated Skin

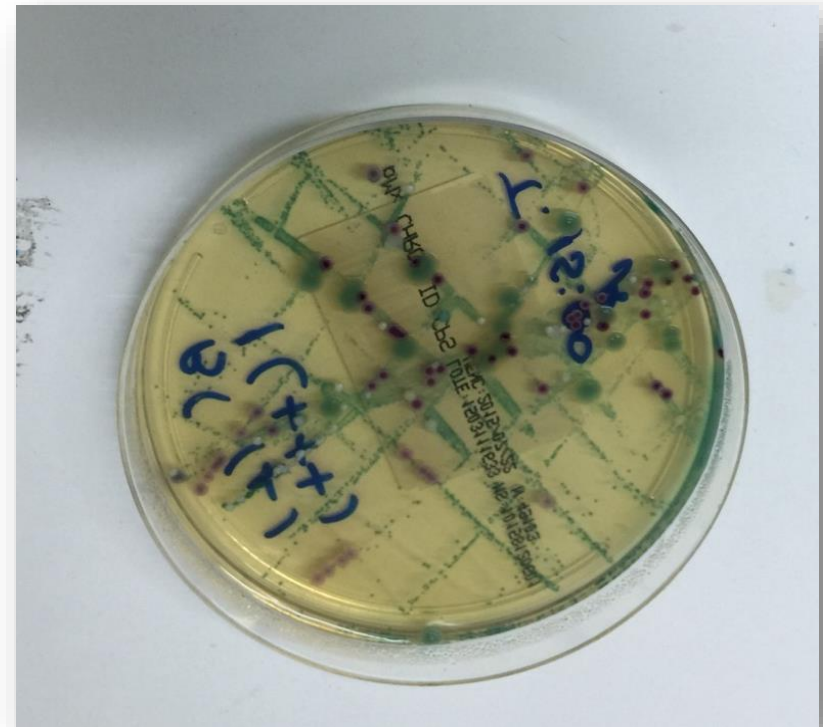
Tilapia Fish Skin
Irradiated 30kGy



	Máx. Carga (N)	Deformação à tração em Máx. de Carga (%)	Carga em quebra (N)	Esforço à tração em quebra (N/mm ²)	Extensão à tração em quebra (cm)
1	36,293	30,358	12,471	1,134	3,09
2	23,941	21,592	17,002	1,619	2,16
3	87,157 4	25,483	72,525	7,883	2,62
4	24,629	23,117	23,299	1,820	2,34
Coef. Variação	69,70203	15,24125	88,82432	102,51455	15,88904
Máx	87,157	30,358	72,525	7,883	3,09
Média	43,005	25,137	31,324	3,114	2,55
Mín	23,941	21,592	12,471	1,134	2,16
Desv. Padrão	29,97529	3,83126	27,82375	3,19242	0,40580

MICROBIOLOGY

- Microbiological Testing In Every Stage – Negative for Gram+, Gram- and Fungi.
- 4 Stages – 2 Glycerol, 1 Chlorhexidine And 1 Applying Skin.



Microbiological Testing



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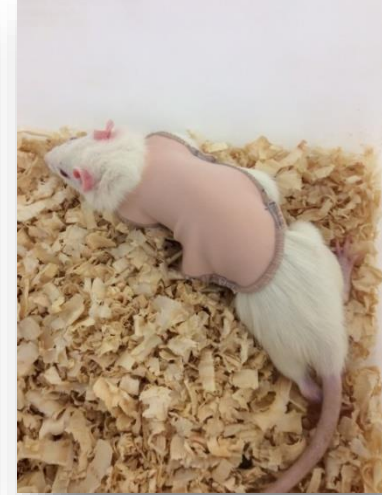
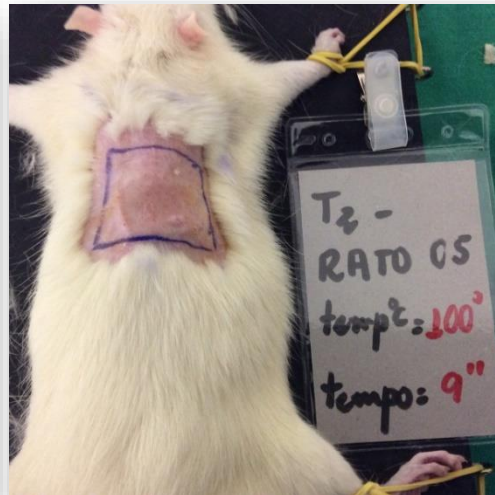
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Testing Appliance Of Tilapia Fish Skin to 40 Wistar Rats

CEUA Approval Under Register 48/2016

PUBLISHED BY SBCP JOURNAL

TESTING IN WISTAR RATS





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MACROSCOPIC EVALUATION

- Wound Measurement;
- Borders Retraction / Crust Formation;
- Rats Were Kept Alive Until 22nd day of P.O.

CONCLUSION

Good outcome from the appliance of tilapia fish skin to the wounds was obtained as regarding adherence to rat's skin and **wound healing improvements: absence of exudate, crusts and hyper granulation.**

Animal Skin Bank – 1st In Brazil



- ISO 5 And 7
- Decontaminated & Sterilized Room



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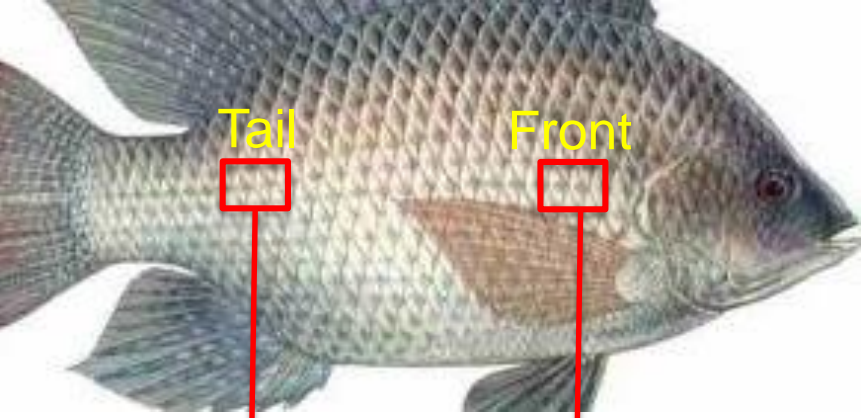
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Evaluation Of Antimicrobial Peptides (AMPs) On The *Oreochromis niloticus* Skin.

Does skin from Tilapia (*O. niloticus*) express β -Defensin,
Piscidin, Hecpcidin, and IL-1 β ?

Methodology: Skin samples from the front and tail portion of *Oreochromis niloticus*

Evaluation in two portions



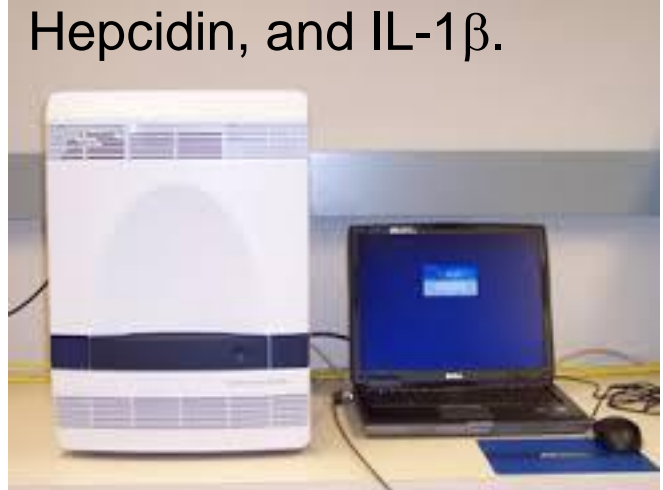
1ml of RNA later- Stabilization immediately stabilizes RNA .



RNA later- Reagent immediately stabilizes RNA .

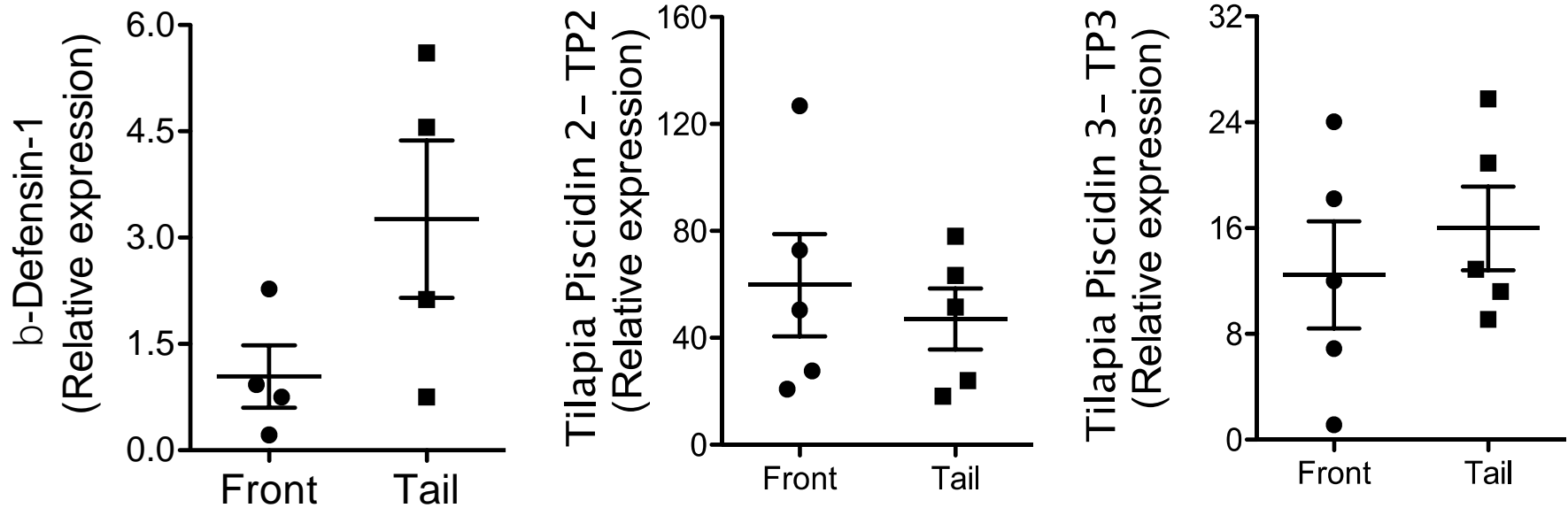
RNA extraction with Trizol reagent.

Real Time qPCR –
Primers: β -defensin1, Piscidin (2 and 3), Hepcidin, and IL-1 β .



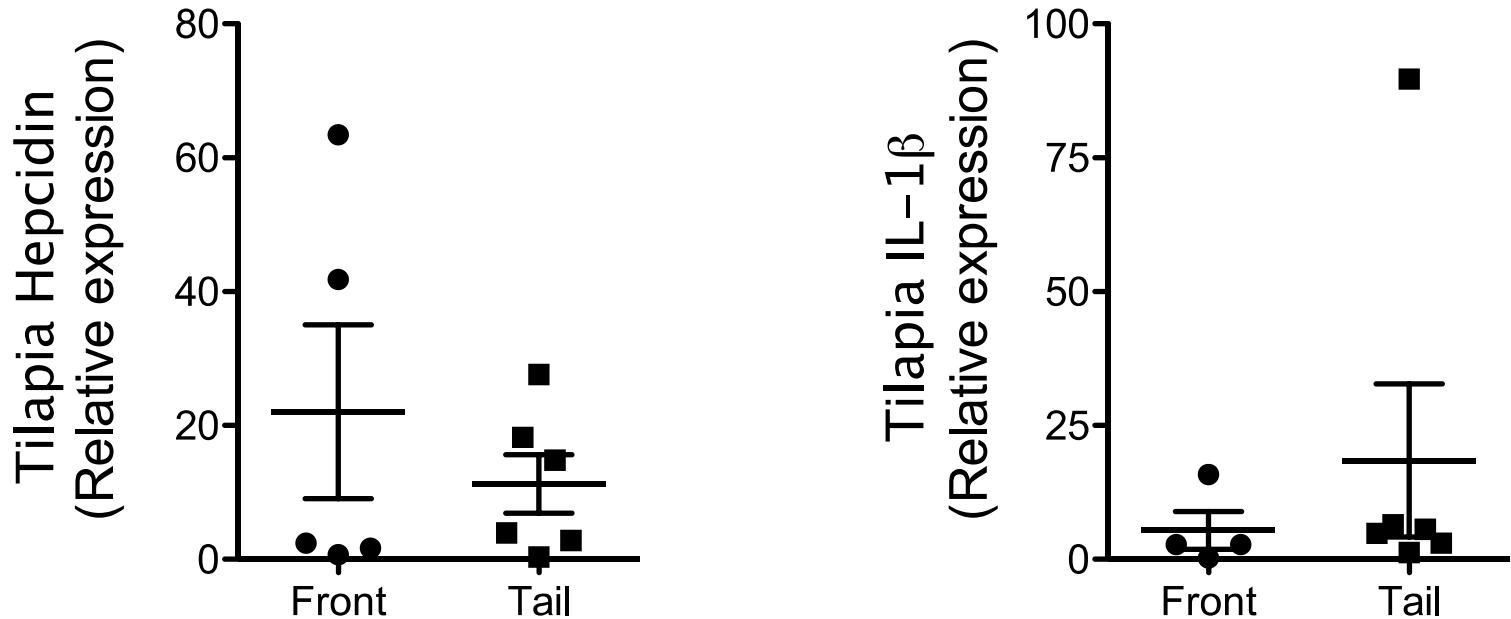
Synthesis of DNA from RNA - cDNA

β -Defensin 1, TP2 and TP3 relative expression on skin from *Oreochromis niloticus*.



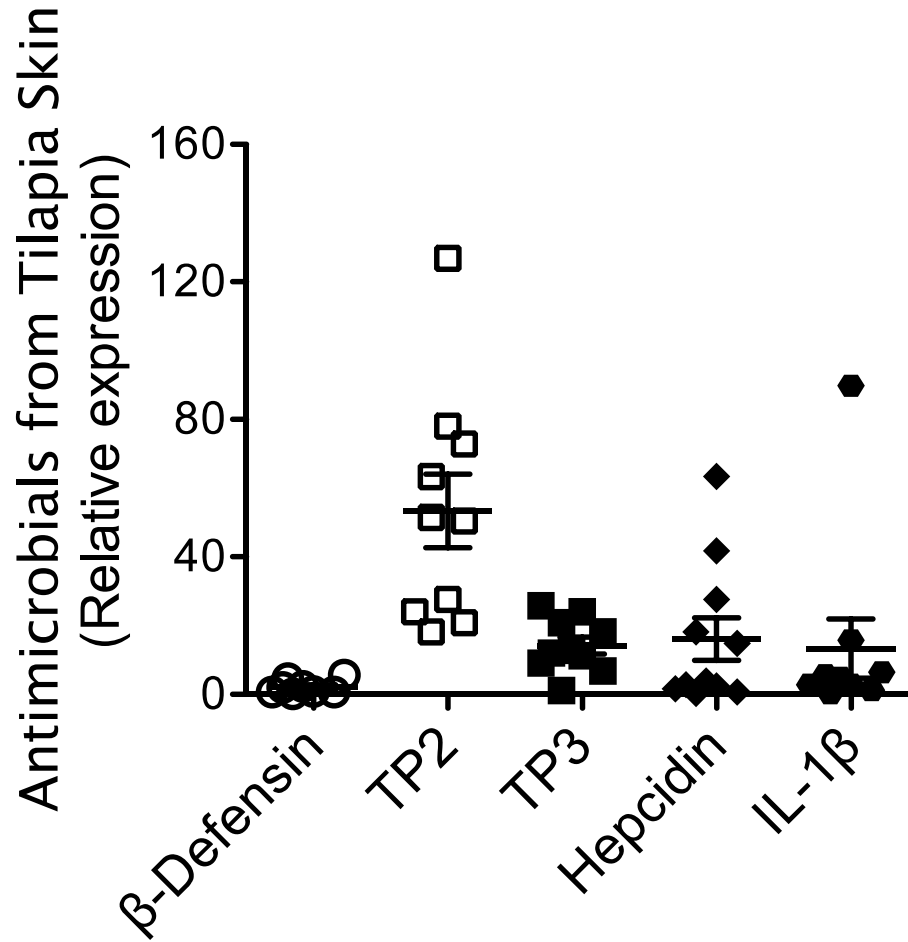
- Relative expression to β -actin (housekeeping gene; constitutive gene).
- No statistically significant difference between two groups (front and tail), n=4-5.

Hepcidin and IL-1 β relative expression on skin from *Oreochromis niloticus*



- Relative expression to β -actin (housekeeping gene; constitutive gene).
- No statistically significant difference between two groups (front and tail), n=4-5.

Overview results of antimicrobials on natural skin from *Oreochromis niloticus*.





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Next steps:

- Antimicrobial evaluation on skin after radiosterilization;
- Protein analysis of the antimicrobial peptides in the skin before and after irradiation;
- Microbicidal evaluation of peptides from *O. n. n. n.* skin.



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Clinical Trial

Tilapia Fish Skin

X

SSD

MORE THAN 100 PATIENTS

Phases II and III (In Progress)



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A1

Superficial Partial Thickness Burns Outpatient

A₁ FP 110353

Healing Time – 09 days

No dressing changes



A₁ AR 311278

Healing Time – 10 days

No skin replacement



A1 MD 250172

Healing Time – 10 days
Skin replacement - 01



A₁ PS 261279

Healing Time – 10 days

No skin replacement



A₁ YS 120397

Healing Time – 12 days



A1 MS 141193

Healing Time – 11 days

Skin replacement - 01



A₁ FR 010176

Healing Time – 09 days

No skin replacement



A₁ JS 300793

Healing Time – 10 days

No skin replacement

No analgesics



A₁ AS 231275

Healing Time – 10 days

No skin replacement



A₁ MN 130967

Healing Time – 11 days

No skin replacement



A1 AP 130167

Healing Time – 09 days

No skin replacement



A1 AP 130167

Healing Time – 09 days

No skin replacement



A₁ LR 090976

Healing Time – 11 days

No skin replacement



A₁ FR 050492

Healing Time – 11 days

No skin replacement

Both legs





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B1

Deep Partial Thickness Burns Inpatient

B₁ MS 050880

Healing Time – 12 days

Skin replacement - 01



B₁ GS 180180

Skin replacement - 01



B₁ GS 180180

Healing Time – 10 days

Dressing changes under anesthesia - 02



B₁ MH 180773

Healing Time – 10 days

No dressing changes



B₁ MH 180773

Early deambulation



B₁ AS 210171

Healing Time – 09 days

No skin replacement



B₁ AS 210171

Skin replacement - 01



B₁ EC 221087

Healing Time – 11 days

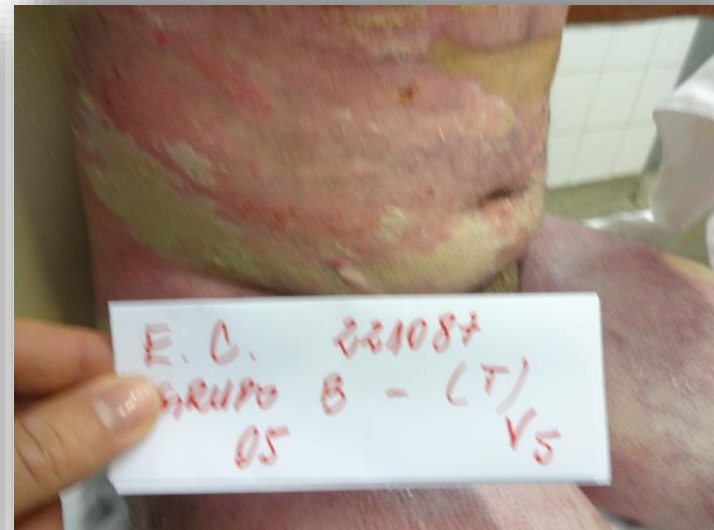
No skin replacement



B₁ EC 221087

Healing Time – 11 days

No skin replacement



B₁ EC 221087

Skin replacement - 03

Debridements - 03

Healing Time – 18 days





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C1

Deep Partial Thickness Burns Inpatient

C₁ JB 150781

Skin replacement – 03



C₁ JB 150781

Healing Time – 18 days

Dressing changes under anesthesia - 04



C1 AS 021082

Skin replacement – 01



C1 AS 021082

No analgesics during 10 days



C₁ AS 021082

Dressing changes under anesthesia– 02

Healing Time – 17 days





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D1

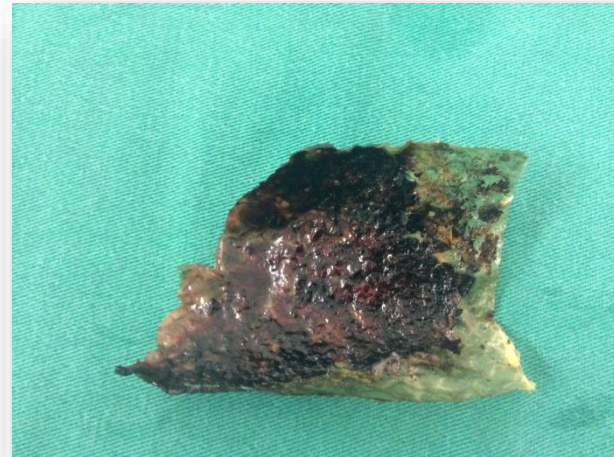
Full Thickness Burns



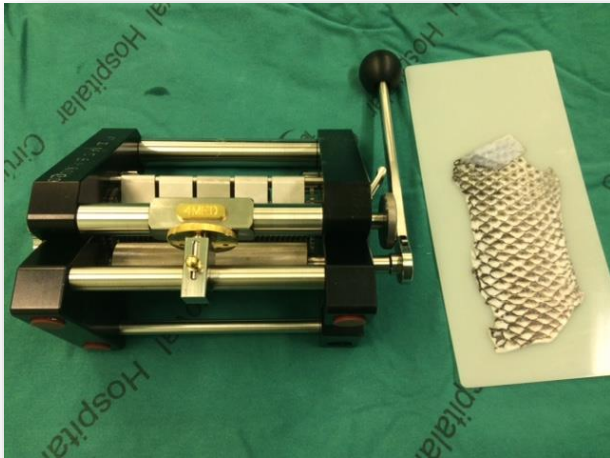




Donor site



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BENEFITS

- Good Adherence To The Wound, Reducing Fluid Loss
- No External Contamination;
- Reduced Number Of Dressing Changes;
- Reduce Pain(30% to 50%) And Costs (57%) Compared To SSD
- Absorption of secretions (Like a Foam);
- No Dirty Dressing Or Bad Odor;
- Reduce In Healing Time(2,5 days);
- Lower Risk of Infection (Peptides) ?



- 1st Brazilian Xenograft.
- 1st Biological Dressing From Aquatic Environment, In The World, To Treat Burn Victims (Clinical Trial)
- Brazil Pays Off A 50 Year Scientific Debt.
- Survey's Future...
- Midia Impact

The Following New York Post Video had over 35 Million Views and received more than 11 Thousand Comments.





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“If one dreams on his own it is just a dream;
if many dream together it is the beginning
of a new reality.”

- Miguel de Cervantes -



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THANK YOU