"stato della ricerca sulla sordità"

Sabato 24 maggio 2014

Prevenzione e riparazione del danno uditivo: cellule staminali e non solo

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Prof. Alessandro MARTINI

"STATO DELLA RICERCA SULLA SORDITÀ"

Ad oggi

Impianti cocleari

Somministrazione di farmaci

Genetica diagnostica

pratica clinica
vs
ricerca biomedica

Futuro:

Cellule staminali

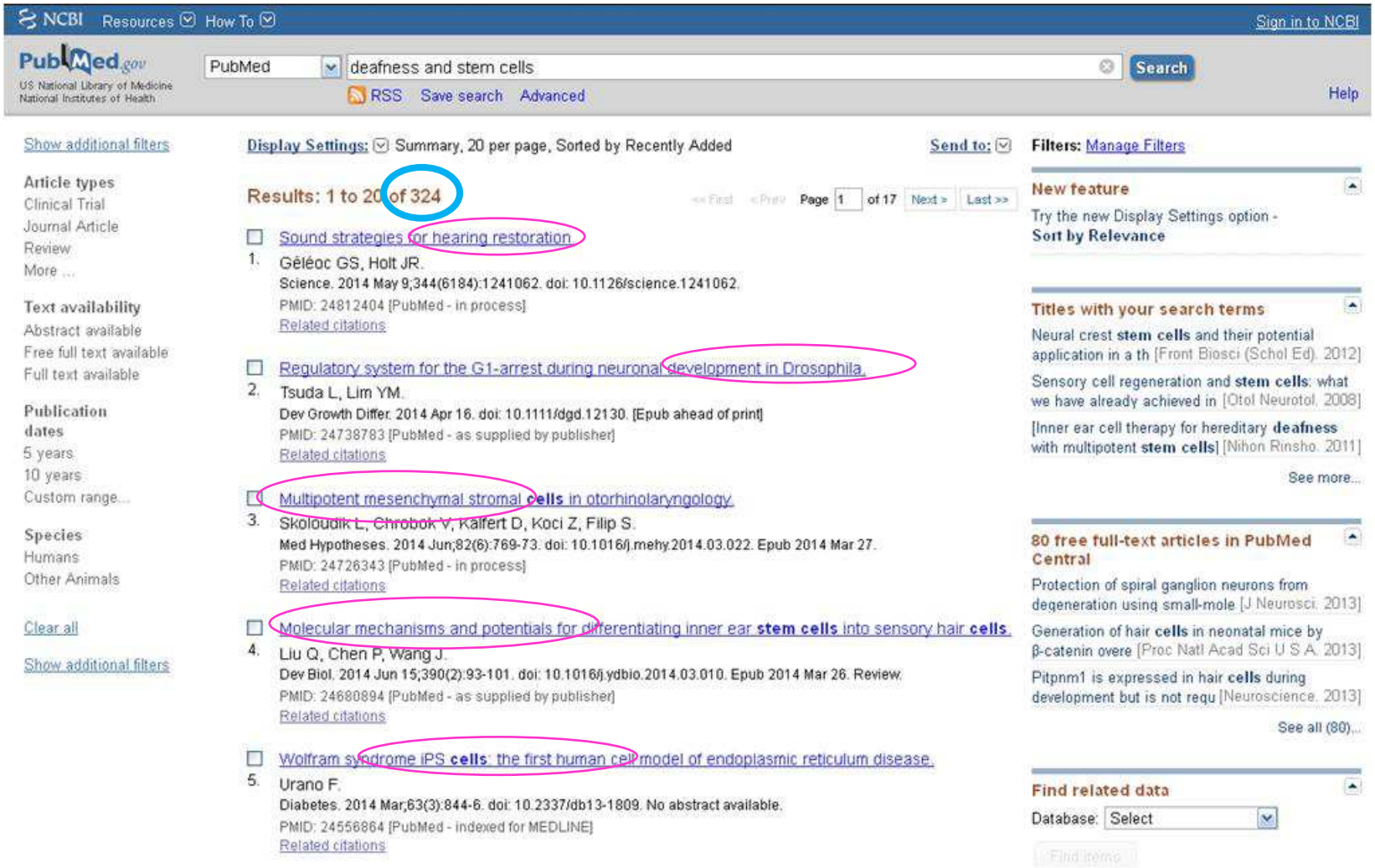
Sviluppo di nuovi farmaci

Nanotecnologie

Terapia genica

ricerca biomedica
vs
pratica clinica

"STATO DELLA RICERCA SULLA SORDITÀ"



CELLULE STAMINALI

1. **Embryonic stem cells**,

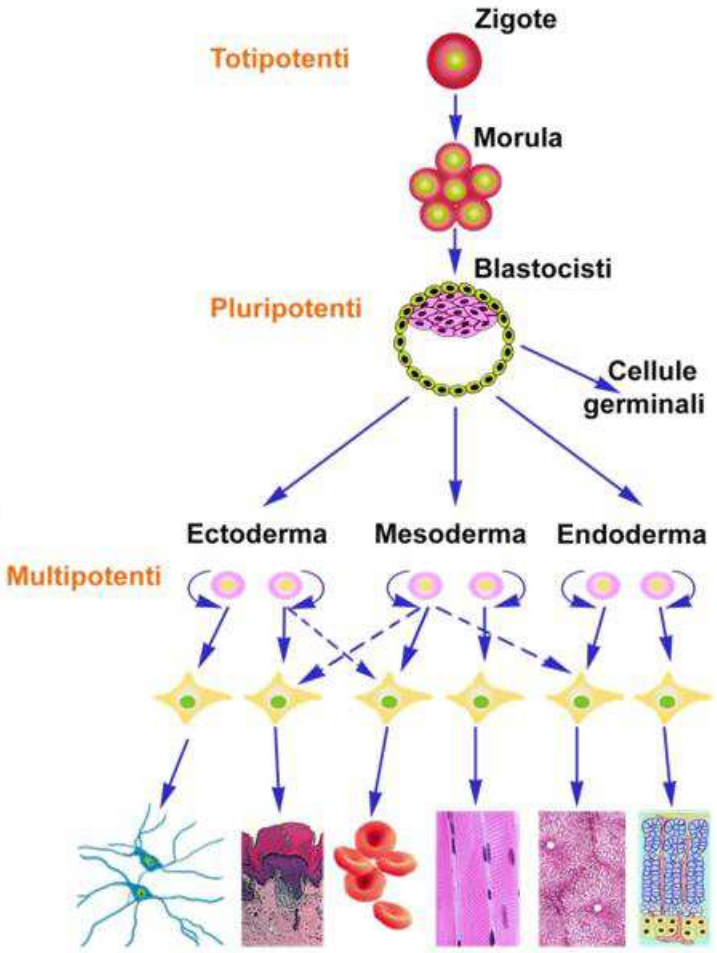
   derivano dalle cellule germinative di embrioni di 4-5 gg

1. **Iduced pluripotent stem cells**,

   tessuti cellulari che sono stati ingegnerizzati per regredire allo stato di cellula staminale

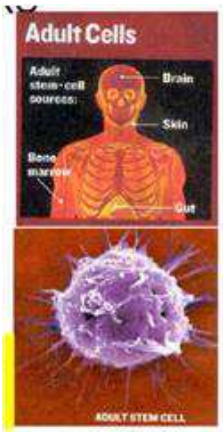
1. **Adult stem cells**,

   derivano dai tessuti adulti

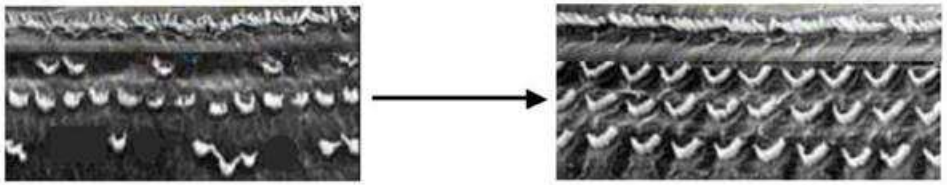


CELLULE STAMINALI

1. Cellule staminali sono state identificate nell'organo vestibolare di ratto
2. E nel nervo cocleare di Guinea pigs e uomo

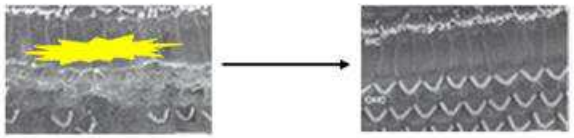


Possibili scenari per la rigenerazione dell'epitelio ciliato



1. trapiantare di cellule staminali nella coclea

2. "risvegliare" cellule staminali endogene, se presenti



3. transdifferenziare cellule di sostegno in ciliate

CELLULE STAMINALI

Cell Transplantation, Vol. 17, pp. 665–678, 2008
Printed in the USA.

0963-6897/08 $90.00 + .00
E-ISSN 1555-3892
www.cognizantcommunication.com

Cochlear Repair by Transplantation of Human Cord Blood CD133⁺ Cells to *Nod-Scid* Mice Made Deaf With Kanamycin and Noise

Roberto P. Revoltella,* Sandra Papini,* Alfredo Rosellini,* Monica Michelini,* Valeria Franceschini,† Andrea Ciorba,‡ Lucia Bertolaso,‡ Sara Magosso,‡ Stavros Hatzopoulos,‡ Guiscardo Lorito,‡ Pietro Giordano,‡ Edi Simoni,‡ Emanuela Ognio,§ Michele Cilli,§ Riccardo Saccardi,¶ Serena Urbani,¶ Rosemary Jeffery,# Richard Poulsom,# and Alessandro Martini‡

1) MODELLO ANIMALE: 70 topi NOD-SCID di 2 mesi di età
(Charles Rivers, Laboratories Clinical, UK)

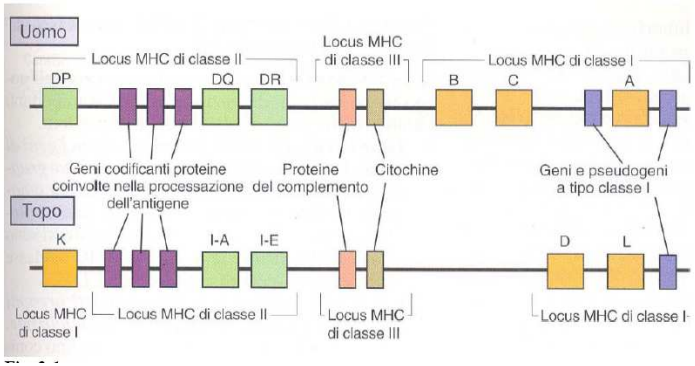
2) DANNO : RUMORE (105 dB SPL; 4 hrs)
AMINIGLICOSIDI (Kamicina: 700 mg\kg\48 hrs x14gg)

3) SOMMINISTRAZIONE SISTEMICA **(IV)**
DI CELLULE STAMINALI (HCBSCS)

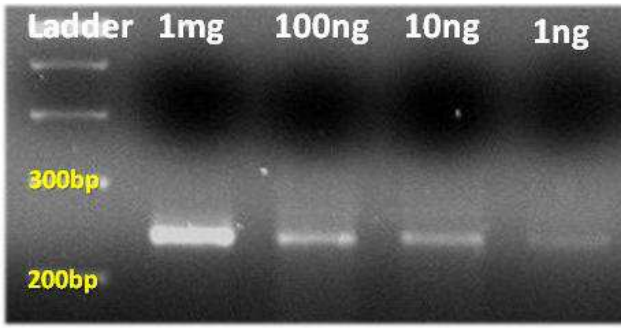
CELLULE STAMINALI

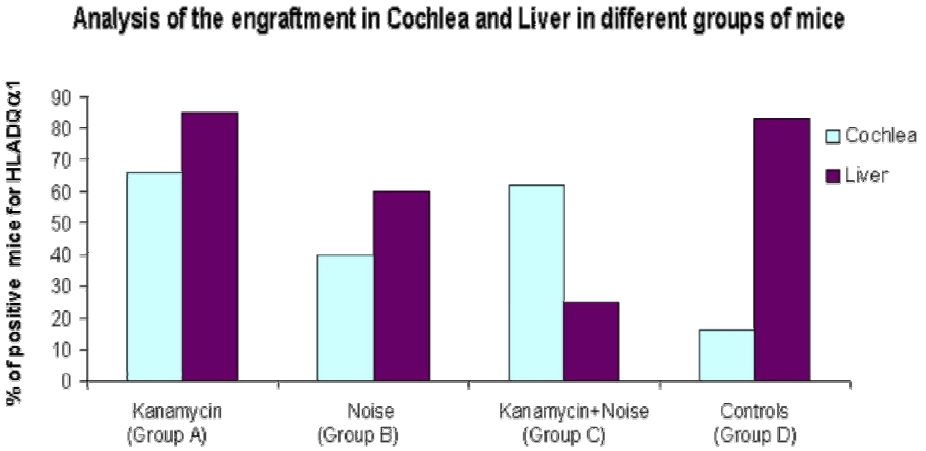
Analisi Molecolare (PCR) : HLA-DQ

MHC di II° classe sono espressi in alcuni precursori mieloidi, in alta percentuale nei monociti/macrofagi, in cellule di langerhans della cute



Sensibilità della PCR HLA-DQa1:

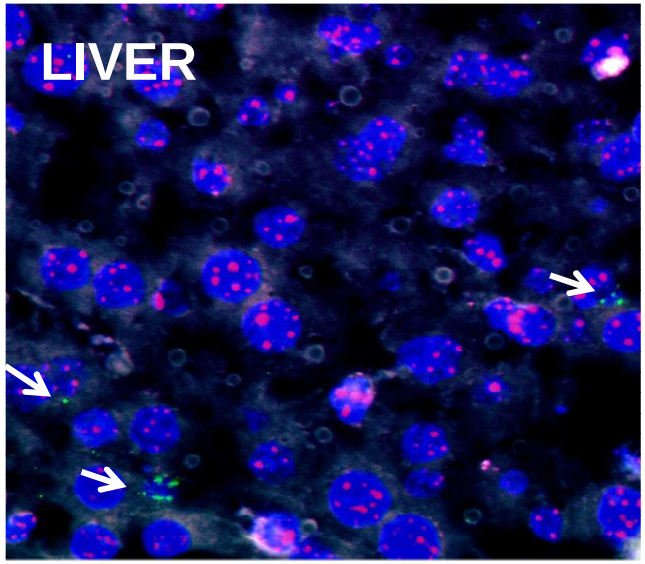




CELLULE STAMINALI

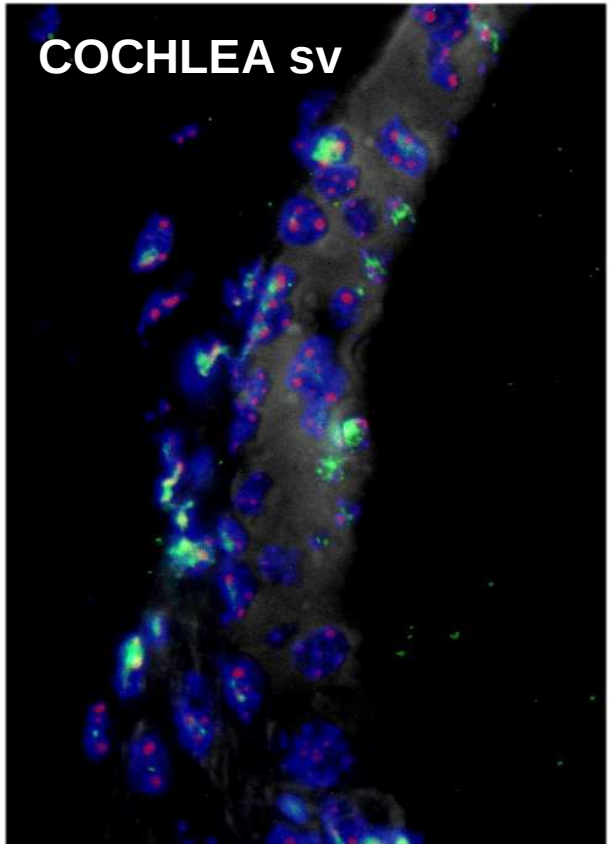
HOMING E INTEGRAZIONE NEL TESSUTO

FISH (DUAL-COLOR)

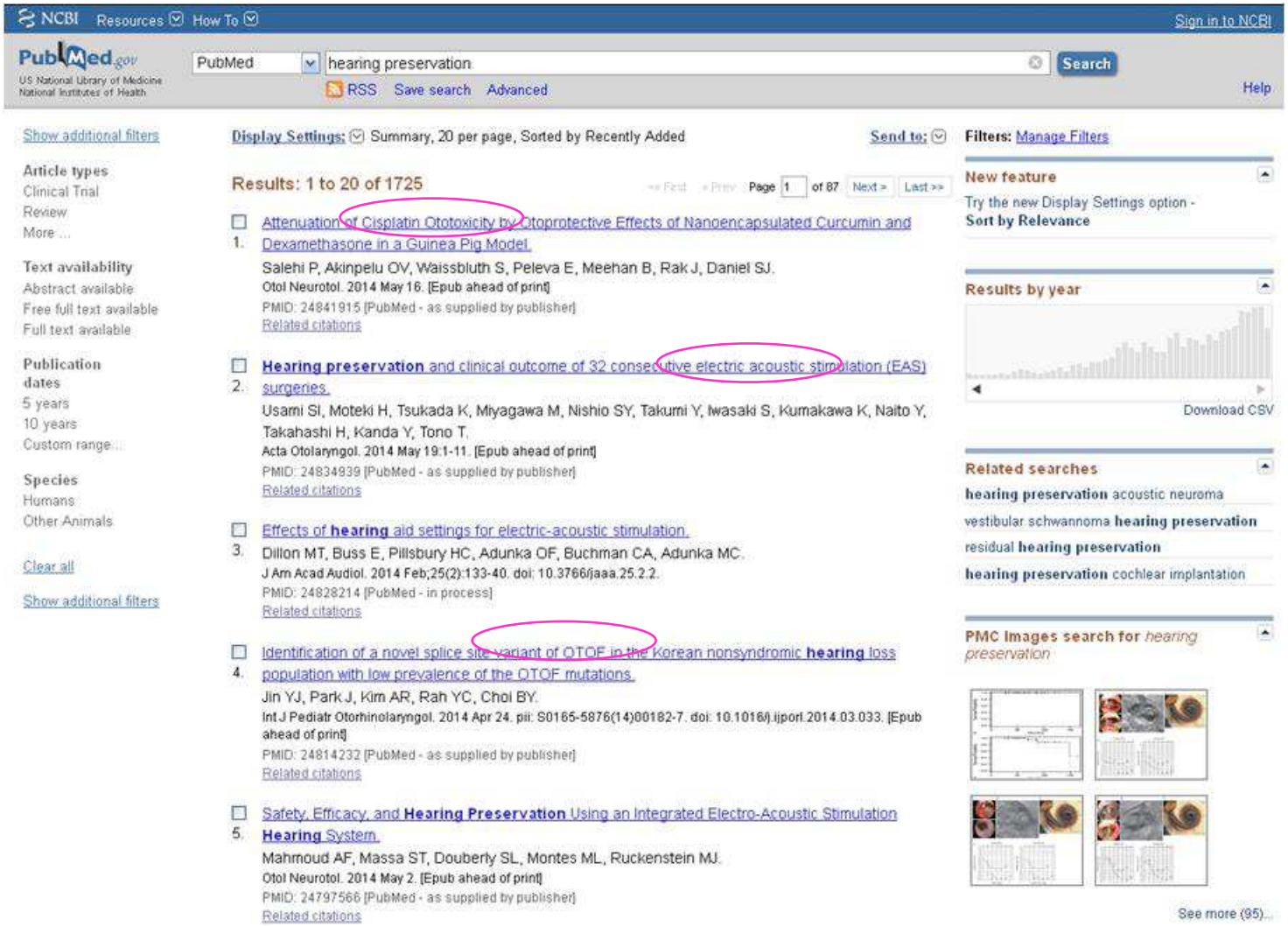


Centromeri murini

Centromeri umani



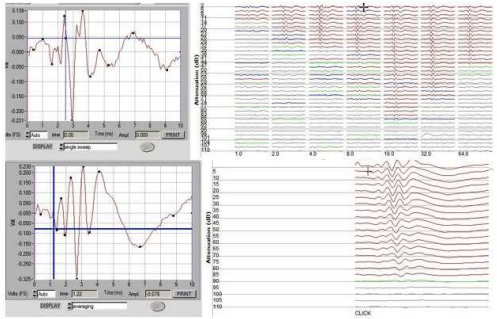
"STATO DELLA RICERCA SULLA SORDITÀ"

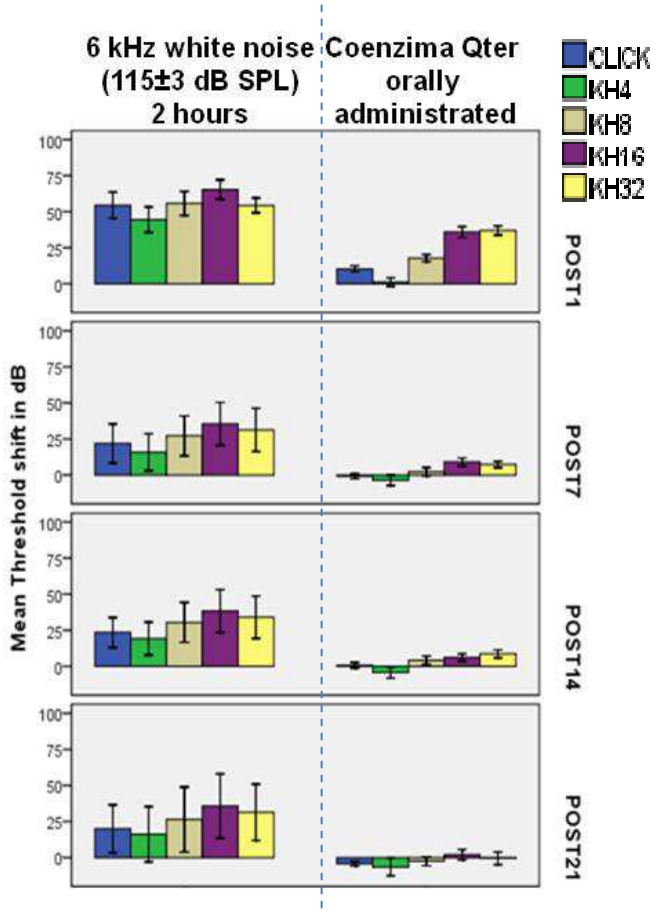


PREVENZIONE DAL DANNO DA RUMORE

MODELLO ANIMALE: RATTO

VERIFICA DELLA FUNZIONE UDITIVA:
ABR thresholds

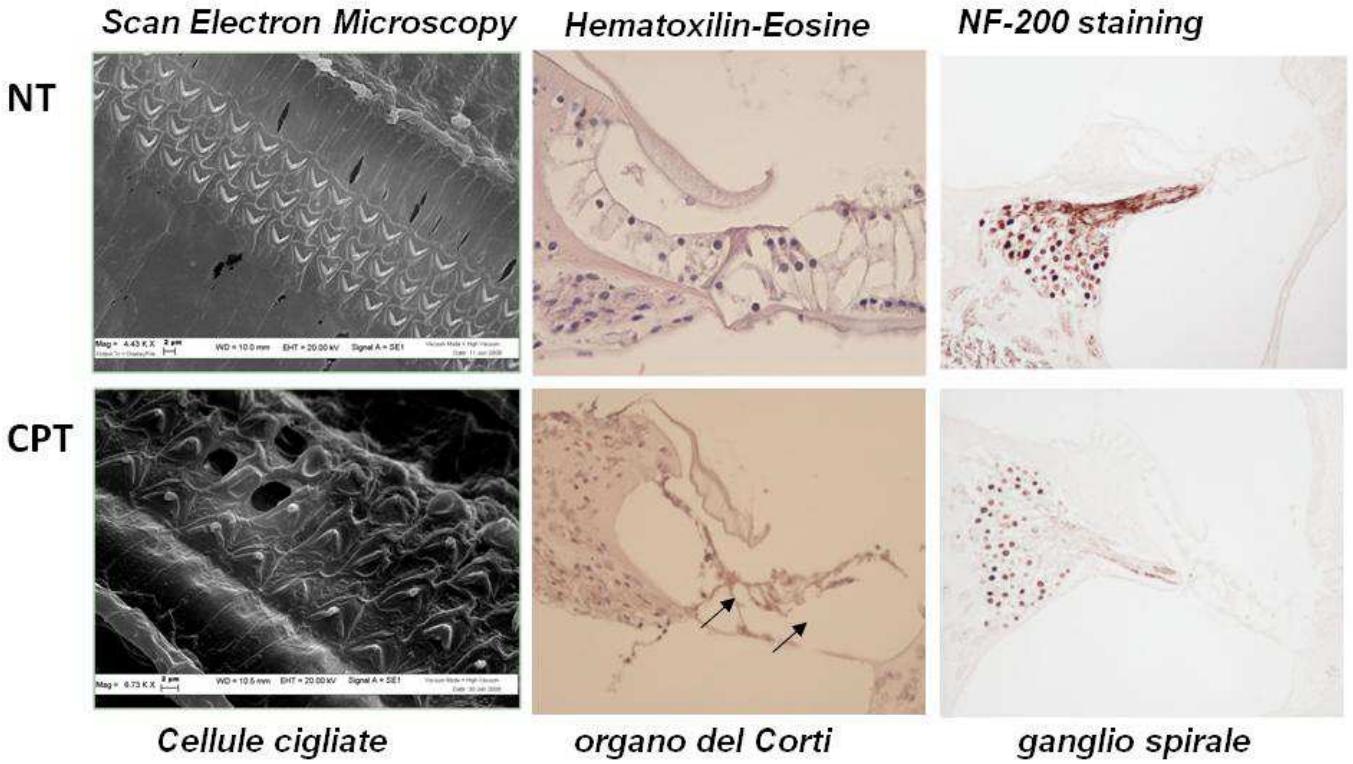




OTOTOSSICITÀ DEL CISPLATINO

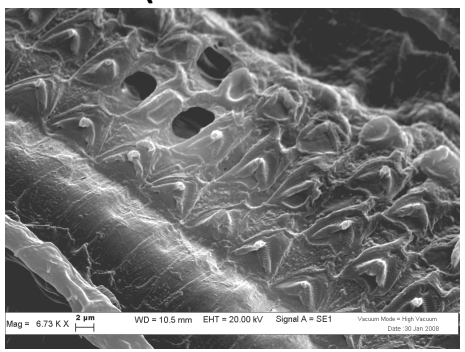
MODELLO ANIMALE: RATTO

TRATTAMENTO: IP 16 mg/kg

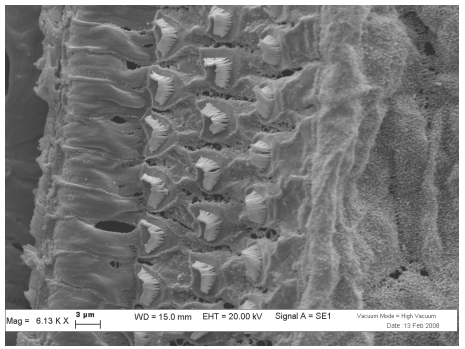


PREVENZIONE DAL DANNO OTOTOSSICO

CISPLATINO (CONTROLLO POSITIVO)



DEXAMETASONE + CISPLATINO



Effetto PROTETTIVO

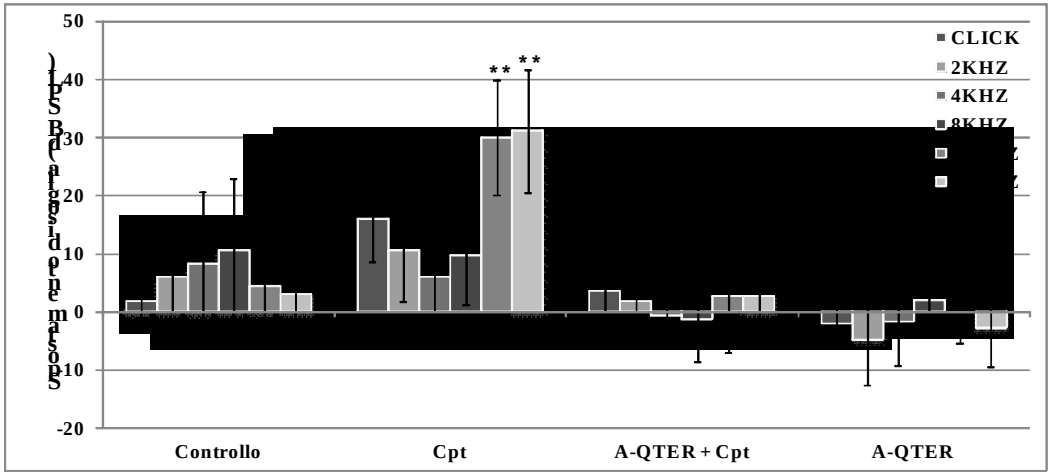
del DEXAMETASONE

nei confronti del CISPLATINO

Effetto PROTETTIVO

del Coenzima Q10

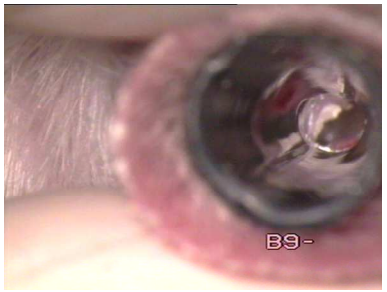
nei confronti del CISPLATINO



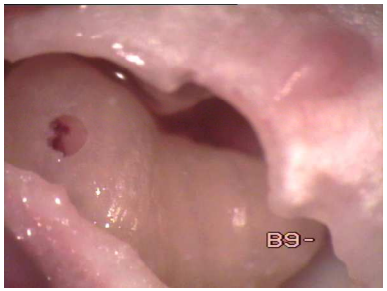
COME APPLICARE I FARMACI

- APPLICAZIONE DIRETTA
- CONIUGATI A NANOPARTICELLE

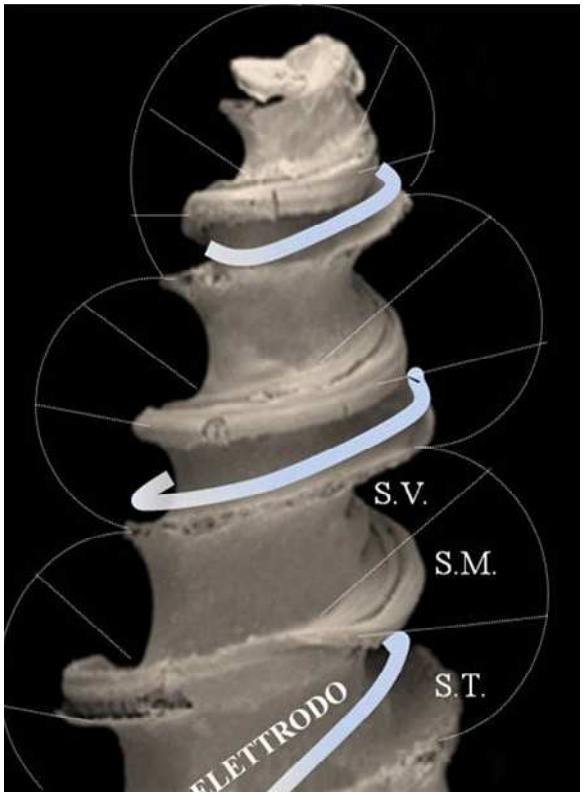
via INTRA-TIMPANICA



via INTRA-COCLEARE



IMPIANTO COCLEARE



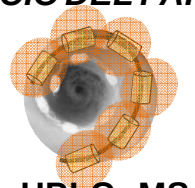
APPLICAZIONE DIRETTA e IMPIANTI COCLEARI

Progetto:
EVALUATION OF THE CAUSES OF COCHLEAR DAMAGE AFTER IMPLANTATION AND THE PROTECTIVE CAPACITY OF A DEXAMETHASONE-RELEASING ELECTRODE

MODELLO ANIMALE

GUINEA PIGS

RILASCIO DEL FARMACO

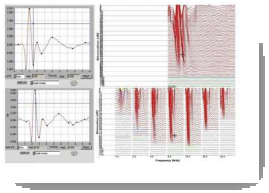


HPLC - MS

IMPIANTO BILATERALE

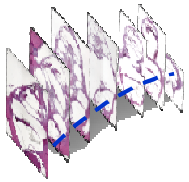
COCLEOSTOMIA

CAPACITÀ UDITIVA



Compound Action Potential

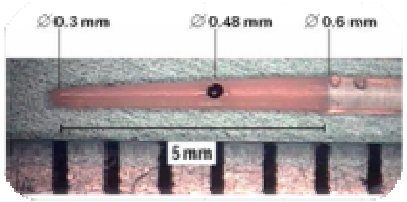
DANNI TISSUTALI

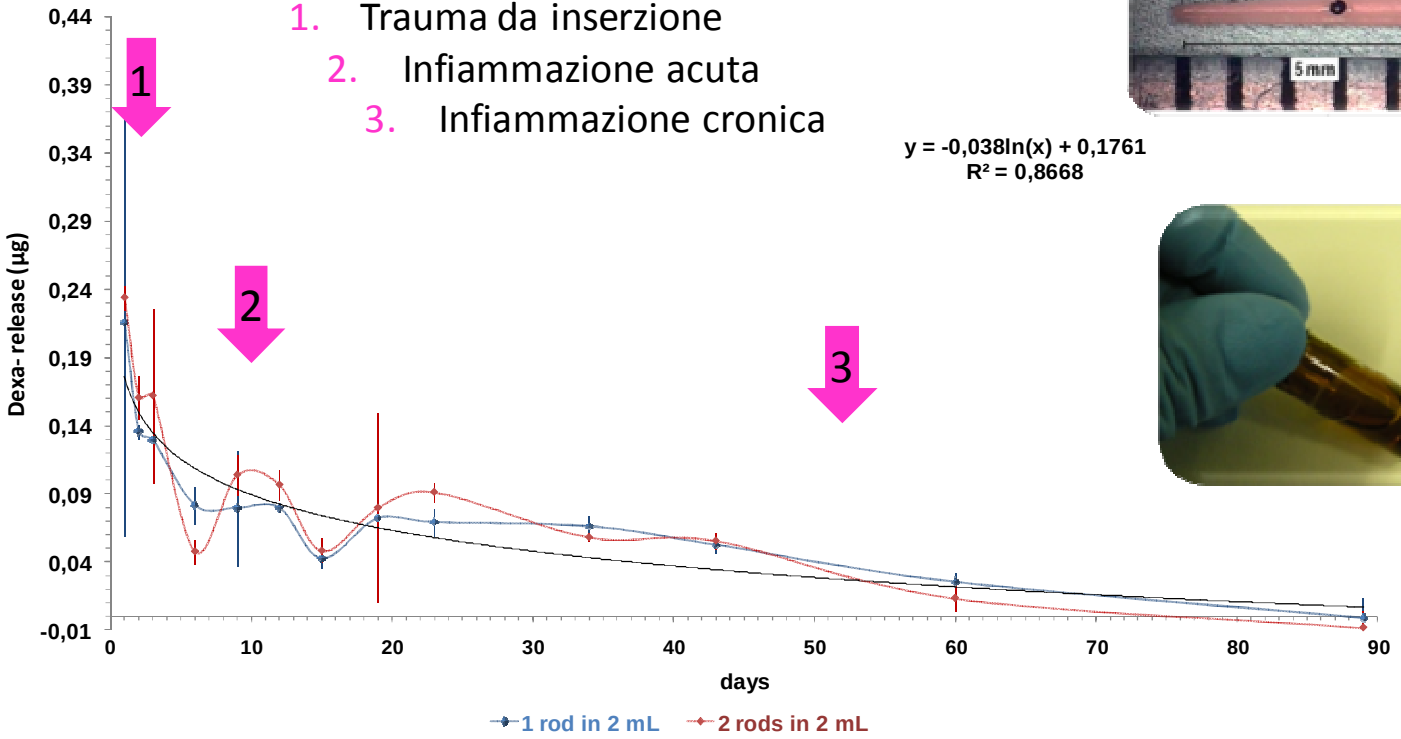


ISTOLOGIA

APPLICAZIONE DIRETTA e IMPIANTI COCLEARI

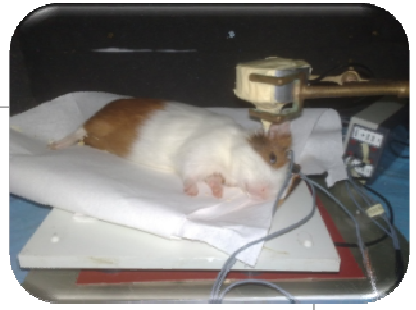
1. Trauma da inserzione
2. Infiammazione acuta
3. Infiammazione cronica

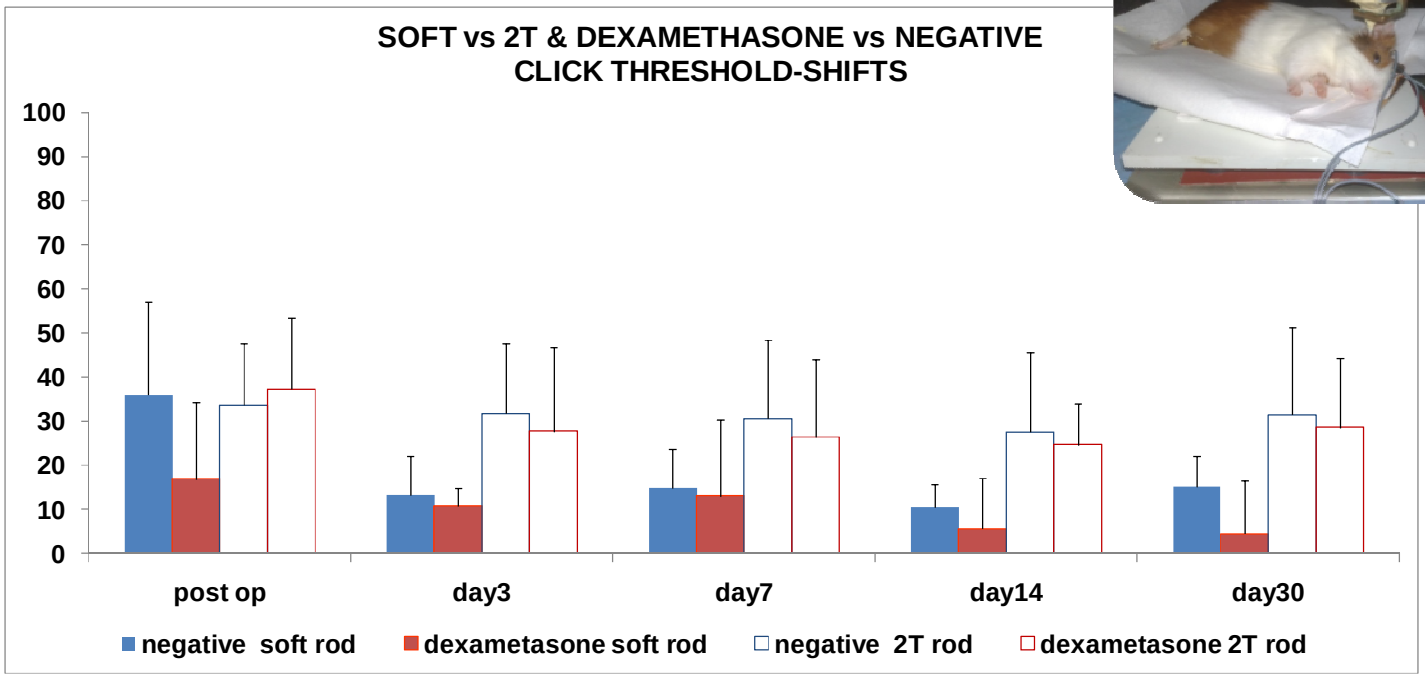




$y = -0{,}038\ln(x) + 0{,}1761$
$R^2 = 0{,}8668$

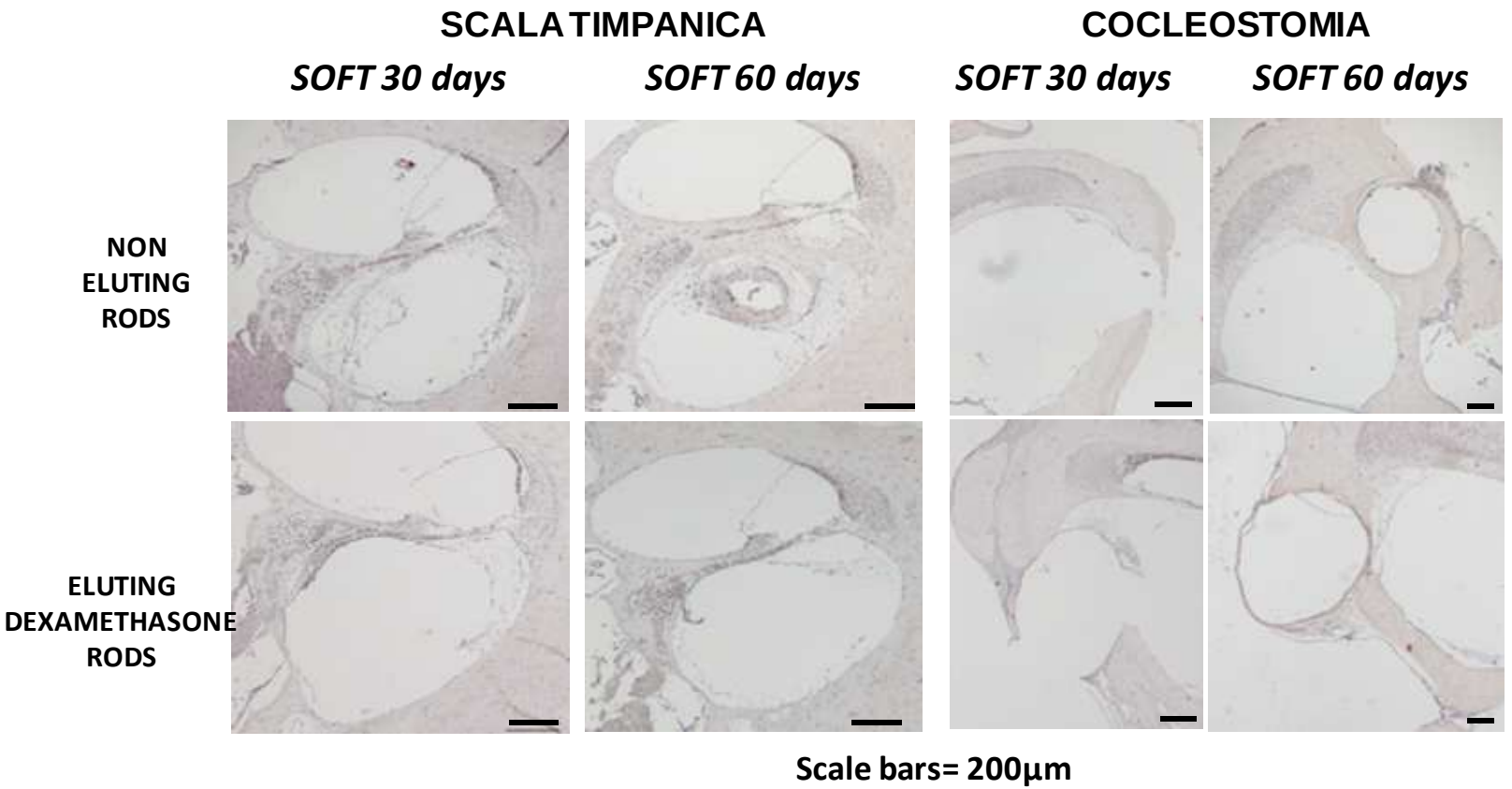
APPLICAZIONE DIRETTA e IMPIANTI COCLEARI





APPLICAZIONE DIRETTA e IMPIANTI COCLEARI

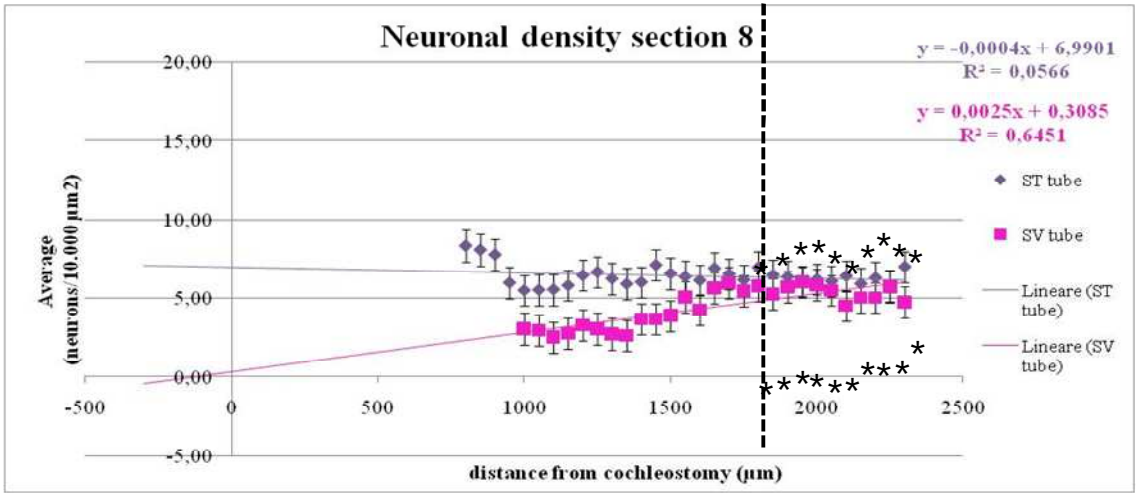
CRESCITA DI TESSUTO FIBROSO



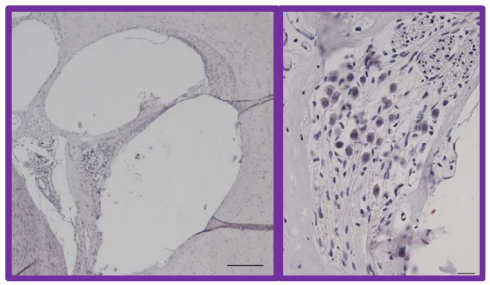
Scale bars= 200µm

APPLICAZIONE DIRETTA e IMPIANTI COCLEARI

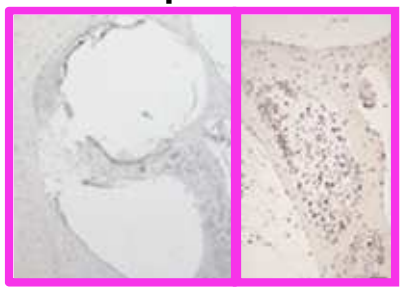
SOFT 60gg **MODIOLUS**



C.I. corretto



C.I. mal posizionato



FARMACI CONIUGATI A NANOPARTICELLE

EU Research proposal: **NMP-2004-3.4.1.5-1**

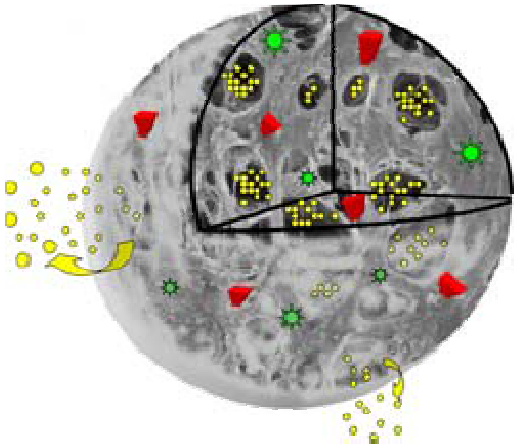
Nanotechnology-vectors for targeted drug and gene delivery

NANOEAR

A questo proposito siamo coinvolti in un progetto europeo, NanoEar, il quale prevede di testare più tipi di nanoparticelle incluse in dendrimeri, micelle o lipidi o complessi polimerici, per costruire un tipo di

NAOPARTICELLA MULTIFUNZIONALE

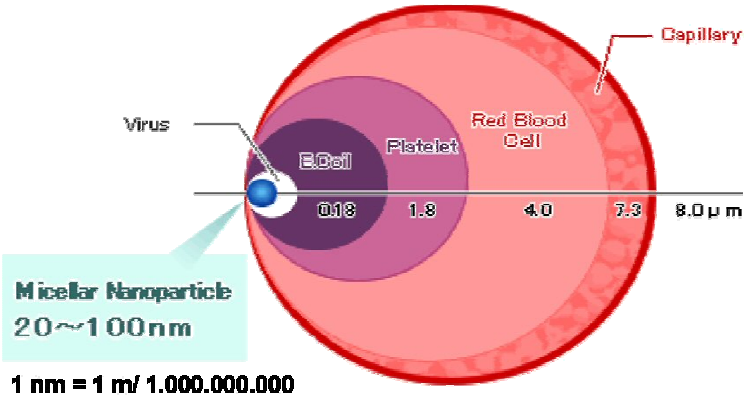
in grado di rilasciare molecole in tessuti specifici dell'orecchio interno



FARMACI CONIUGATI A NANOPARTICELLE

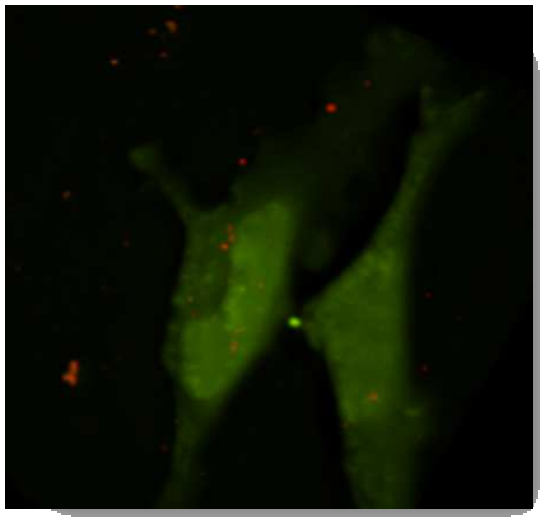
L'utilizzo delle NPs per la diffusione dei farmaci permette di avere

- Aumento della solubilità
- Aumento della biodisponibilità
- risposta terapeutica più veloce
- diminuire le dosi da somministrare



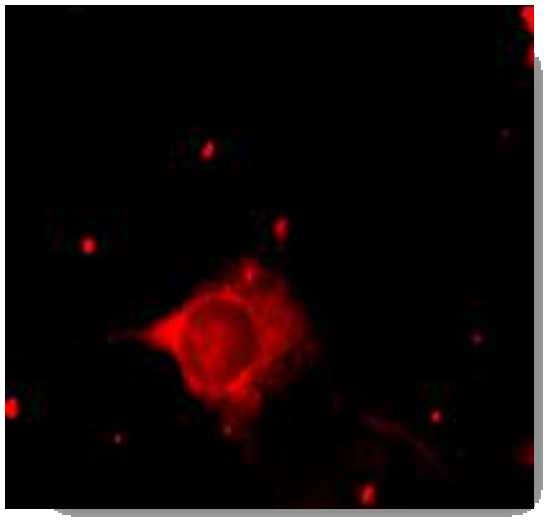
FARMACI CONIUGATI A NANOPARTICELLE

USO DI NPs COME DELIVERY SYSTEM



HeLa

EGFP/HBPL-Texas Red



OC-k3

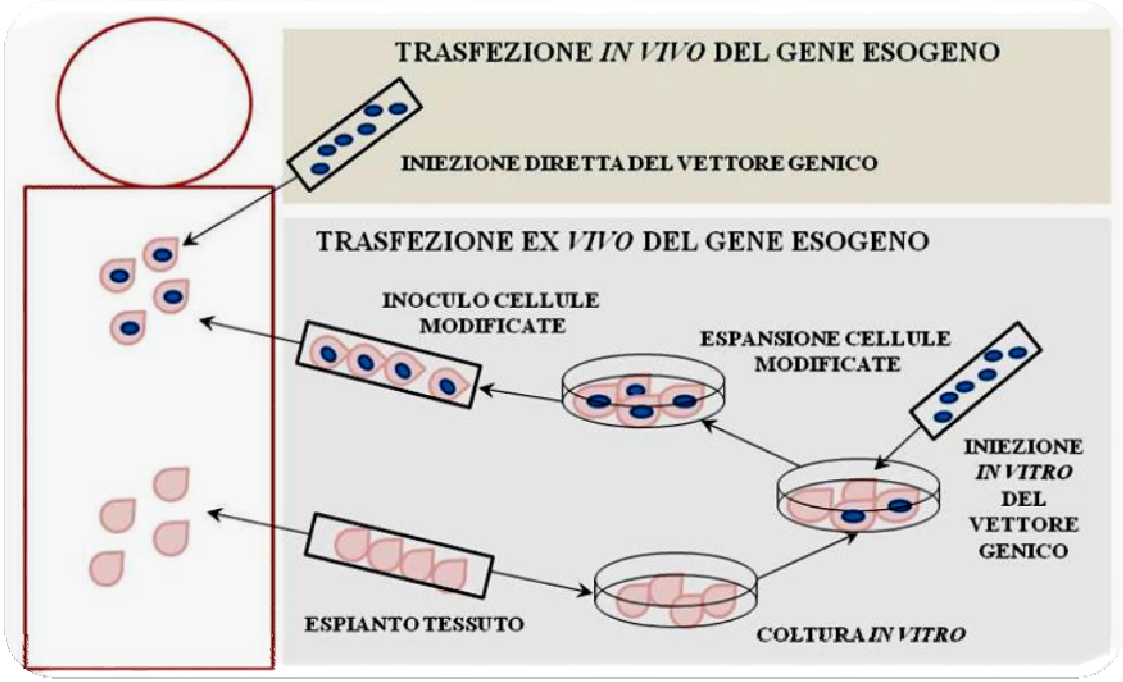
Qdot

Elisa Corbacella, Alessandro Martini e Ilmari Pyykko (2011).
Il rilascio di farmaci nell'orecchio interno basato sulle Nanotecnologie
In: Nano & Biotech in Audiologia e Otologia, a cura di A. Martini e G. Paludetti. ISBN 978 88 7241

TERAPIA GENICA

Vettori di trasporto

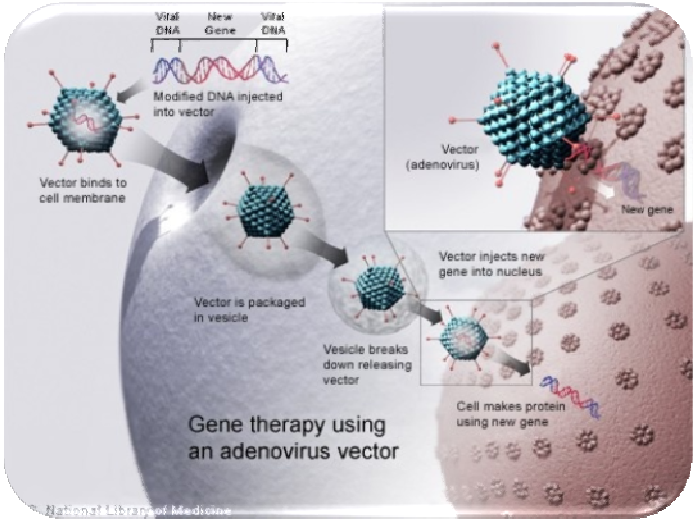
- Virus (es. retrovirus, adenovirus)
- Liposomes
- siRNA
- Sistemi trasponibili



TERAPIA GENICA

VETTORI VIRALI

- Retrovirus
- Adenovirus
- Adeno-associated virus
- Herpesvirus
- Poxvirus



Vantaggi:

- Integrazione stabile del gene

Svantaggi:

- Piccole dimensioni dei geni da trasferire
- Sicurezza
- mutagenesi
- Immunogenicità/potenziale infiammatorio

TERAPIA GENICA

APPLICATA all'orecchio interno

ELSEVIER

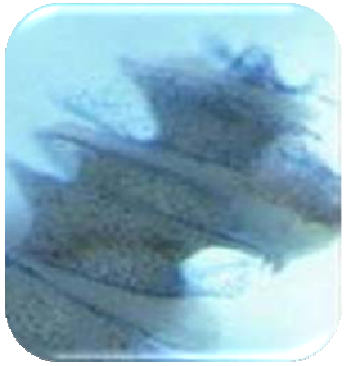
Neuroscience Letters 207 (1996) 137–141

NEUROSCIENCE LETTERS

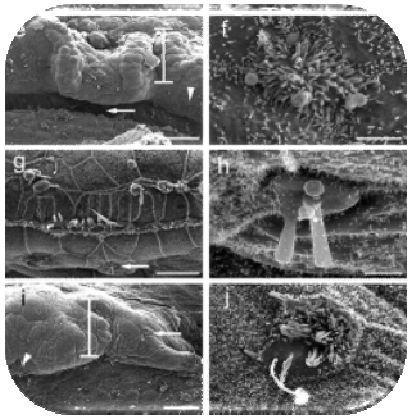
Adenoviral-mediated gene transfer into guinea pig cochlear cells in vivo

Yehoash Raphael[a,*], Juan C. Frisancho[a], Blake J. Roessler[b]

[a]Kresge Hearing Research Institute, Department of Otolaryngology, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-0648, USA
[b]Department of Internal Medicine, University of Michigan Medical School, MSRB MI Room 9303, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-0648, USA



The Journal of Neuroscience, June 1, 2003 · 23(11):4395–4400 · 4395



Brief Communication

Math1 Gene Transfer Generates New Cochlear Hair Cells in Mature Guinea Pigs *In Vivo*

Kohei Kawamoto,[1,2] Shin-Ichi Ishimoto,[1,3] Ryosei Minoda,[1,4] Douglas E. Brough,[5] and Yehoash Raphael[1]

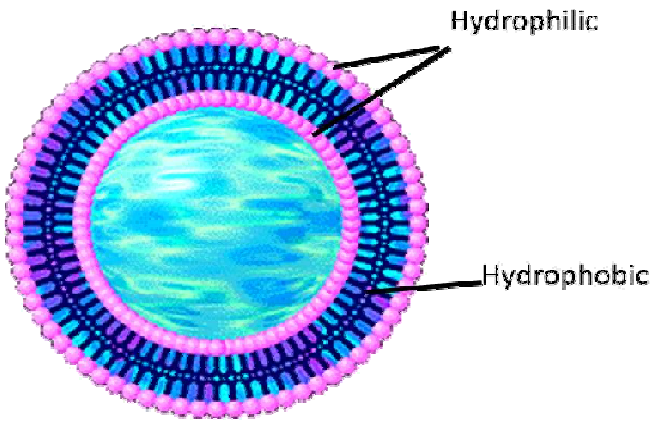
[1]Kresge Hearing Research Institute, Department of Otolaryngology, The University of Michigan, Ann Arbor, Michigan 48109-0648, [2]Department of Otolaryngology, Kansai Medical University, Moriguchi, Osaka, 570-8506, Japan, [3]Department of Otolaryngology, Tokyo University, Bunkyo-ku, Tokyo, 113-8655, Japan, [4]Department of Otolaryngology–Head and Neck Surgery, Kumamoto University School of Medicine, Kumamoto, 860-8556, Japan, and [5]GenVec Inc., Gaithersburg, Maryland 20878

TERAPIA GENICA

VETTORI NON VIRALI

- **Liposomes=lipoplexes**
- **Polycations=polyplexes**
- **siRNA**

Liposomes=lipoplexes



VANTAGGI

- Disegno versatile
- Non-immunogenic e non-tossici
- Prodotti farmaceutici ben caratterizzati e rirpoducibili

SVANTAGGI

- No integrazione nel genoma ospite
- Possibili ripetizioni di somministrazione

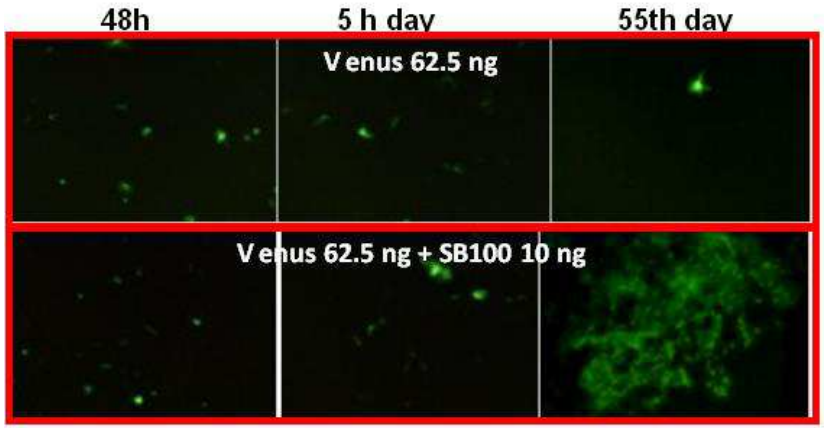
TERAPIA GENICA

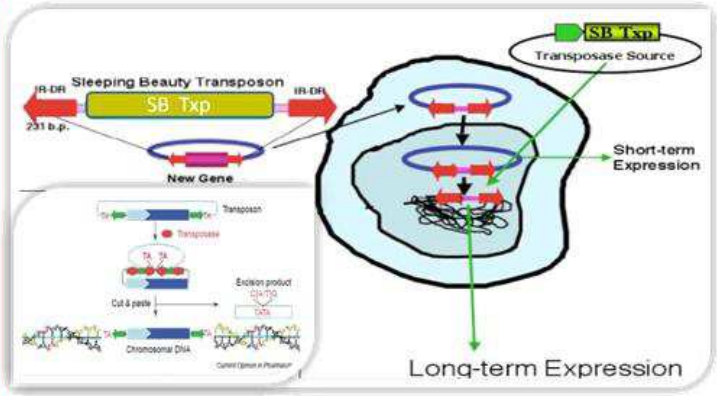
IN VITRO efficienza di trasfezione

Modello *in vitro* hFASC stem cells

con **Sleeping Beauty transposable system**

Efficienza di co - Transfection in hearing fetal stem cells





Laura Astolfi, Valeria Guaran, Alessandro Martini (2011)
Terapia genica per l'orecchio interno: presente e futuro
In: Nano & Biotech in Audiologia e Otologia, a cura di A. Martini e G. Paludetti. ISBN 978 88 7241

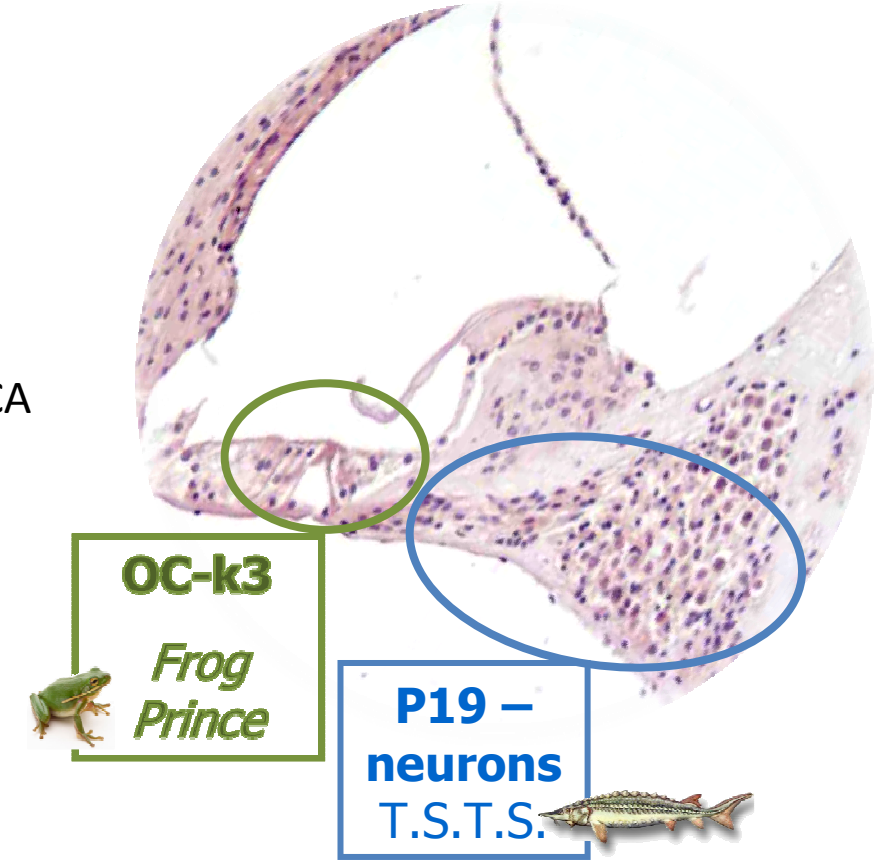
TERAPIA GENICA

FUTURE APPLICAZIONI

-con STEM CELL
in protocolli ex-vivo
verifica dell'integrazione
verifica di dove e come agiscono

- Nuovi SISTEMI DI TERAPIA GENICA

no virus
Integrazioni stabili
differenti target cells



PROSPETTIVE FUTURE

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GRAZIE PER L'ATTENZIONE