



NANOCI

Testing innovative bio-funcionalized compounds for the ear

Sheffield 30. August 2014





NANOCI



Figure 1.3-1. Schematic view of the project workflow and of work package interdependences.





Bioassays

Task 4.1Murine Bioassay

UT (Lead), Duration: M01 – M36

- Screening of neurotrophin-like compounds
- Testing of 3D nanomatrices
- Test electrode array surfaces

Task 3.1: Interface-dependent stimulation patterns UNIBE (Lead), MED-EL M01 – M36

- MEA technology
- To develop coding and signal shaping strategies

Task 6.4: In vivo bioassay with guinea pigs

UT (Lead), MED-EL M01 – M36

- Delivery of 3D nanomatrix into the deafened cochlea
- Chronical implantation
- physiological measurements
- Cochlear histology





Methods











Neurite index = number of intersections x distance



Screening









Screening of TrkB agonists



TrkB agonist	Literature		
LM22A-1	<u>Massa</u> et al. 2010, The Journal of Clinical Investigation		
LM22A-4	<u>Massa</u> et al. 2010, The Journal of Clinical Investigation		
Deoxygedunin	<u>lang</u> et al. 2010, <i>PLOS one</i>		
7,8-Dihydroxyflavone	Jang et al. 2010, PNAS Yu et al. 2013, The Journal of Neuroscience		
7,8,3`-Trihydroxyflavone	Yu et al. 2012, Biochemical and Biophysical Research Communications Yu et al. 2013, The Journal of Neuroscience		





Screening of BDNF mimetics









by Pascal Senn







Methods



see also Poster Frick et al.





Tolerability / Toxicity

	3-D nanomatrices	Handling	Stability	Tolerability
В	Pam-AAAAGGGEIKVAV (Silva et al. Science, 2004) Background	+	+	+
1	BD™ PuraMatrix™ Peptide Hydrogel	+	+	+
2	HydroMatrix [™] Peptide Hydrogel	+	+	+
3	3-D Life Dextran-CD Hydrogel Kit	+	+	+
4	3-D Life PVA-CD Hydrogel Kit	+	+	+
5	3-D Life Dextran-CD + PVA-CD Hydrogel Kit	+	+	+
6	AuxiGel™	-	N. D.	N. D.
7	HyStem [™] -HP Cell Culture Scaffold Kit	+	-	+
8	Corning [®] Matrigel [®] Matrix	+	-	+

 \rightarrow

nanomatrices were highly tolerable and showed no toxicity on growing spiral ganglion neurites.





Tolerability / Toxicity



 \rightarrow The presence of 3-D nanomatrices does not influendce neurite growth







Testing of 3-D nanomatrices modified by different neurotrophic epitopes and/or neurotrophin-mimetics for functionality, biocompatibility and biostability





Nanomatrix attraction and penetration



3-D Life Dextran-CD Hydrogel

 \rightarrow Neurites were shown to grow on the 3-D nanomatrix surface.





Strategies to improve nanomatrix attraction







Strategies to improve nanomatrix attraction







Assay to test electrode array surfaces

Development of MED-EL silicone rings

Direct application into culture chambers









Assay to test electrode array surfaces

MED-EL silicone rings in the spiral ganglion explant culture







SGN culture on MEA

-perform extracellular recordings

-recordings form a population of SGN and not single neurons like in patch clamp -customize electrode surface (material, size, coating)



Physiology department University of Bern





Spontaneous activity of SG auditory neurons in vitro (d18) on MEA



Basal



Data by Stefan Hahnewald, IEB on Tuesday





Response profile upon stimulation from MEA electrode







Patterning approaches to increase recoding efficiency









Patterning approaches to increase recoding efficiency



Neurons nuclei



In vivo experiments



- Deafening procedure
- Histology
- Physiology
- Cochlea-Implant Surgery



Compound Action Potential (CAP)











CAP-Threshold measurement





Müller et al., unpublished





Deafening – Surgical approach



- Retroauricular approach
- Opening the bulla
- CAP-Electrode
- 1 ml syringe + 30G needle to fill the bulla with
- kanamycin/furosemide solution
- After exposure time rinse with Ringer solution



Audiology



- Summary:
 - Group 1 (full dose, full time):
 79% deaf
 - Group 2 (full dose, half time):
 - 100 % deaf
 - Group 3, 4 (half dose/half time):
 - 80%, 53%
 but steady effect
 - Group 5, 6:
 - no hearing loss





Histology









Histology





see Poster Bako et al.























500 µm



20 µm

(45/32645.45)*10⁶= 1378.44 cell/mm²















"Form" like ring at 4 mm •





- Retroauricular approach
- Opening the bulla
- CAP-Electrode
- Cochleostomy
- CI implantation
- Implant-embedding

















100







•Normal hearing guinea pig

CAP response



click

eCAP response



biphase click







100 µm





to be continued ...