

Tables:

WP8

Summary of the recommendations of lay participants in the GAMBA panels:

Area	Citizen panel	Patient panel	CH*
Opportunities			
Important findings for other fields	x	x	x
Improvement/healing	x	x	x
Individually through the use of the body's own stem cells	x	x	
Special opportunities for those under 45 years	x	x	x
Less invasive	x		
Fewer side effects than with medication		x	
Experiments recommended with older stem cells		x	
Better understanding of osteoarthritis and its prevention		x	x
Risks			
Gene therapy, Stem cell research and nanoparticles are risky	x	x	x
Before human application comprehensive risk assessment is necessary	x	x	x
Danger of risks being concealed	x	x	x
Risks are justifiable or opportunities outweigh risks	x	x	
Particular attention should be paid to as yet unknown risks	x	x	x
Complexity of the approach itself	x	x	x
Risk of cancer and risk of death	x	x	x
Effect on the environment or third parties	x		x
Epigenetic influences open	x		x
Undesired immune reactions		x	x
Control of bodily processes difficult		x	x
Quality of the materials and their origins good enough?		x	x
Prior exposure of patients given enough thought?		x	x
Biological materials		x	x
Spreading of manipulated stem cells and growth factors		x	x
Risks must be quantified and solution strategies must be developed		x	x
Ethical Aspects			
Ethics committees must be well balanced regarding members and provided with sufficient time; transparency must be guaranteed, and decisions must be checked	x	x	
Animal experimentation justifiable under certain conditions	x	x	x
Global ethical standards and regulations important	x	x	x
Ethical questions must be checked numerous times	x	x	x
GAMBA = reductionist human image	x		x
Adult stem cells are less critical compared with embryonic stem cells	x		
Enhancement must be discussed	x		x
Informed consent!		x	
Animals suffering from osteoarthritis		x	x
Patient data privacy!		x	
Other aspects			
Further research regarding the cause of primary osteoarthritis necessary	x	x	x
Negative results should also be published	x	x	x
Avoid hasty promises of cures	x	x	
Neutral presentation of the whole range of therapies	x	x	

Area	Citizen panel	Patient panel	CH*
Early dialogue important and desirable as a standard	x	x	
Sufficient funding and access to basic research in all areas and EU member states	x	x	x
Increased transparency and information exchange in research policies	x	x	x
Conflicts of interest must be made clear	x	x	x
Therapy should be effective, affordable and available for all	x	x	

x (bold / in grey) = both special panels, x (normal) = at least one special panel

*Switzerland: mixed panel with 8 patients and 3 citizens

Figures

WP1

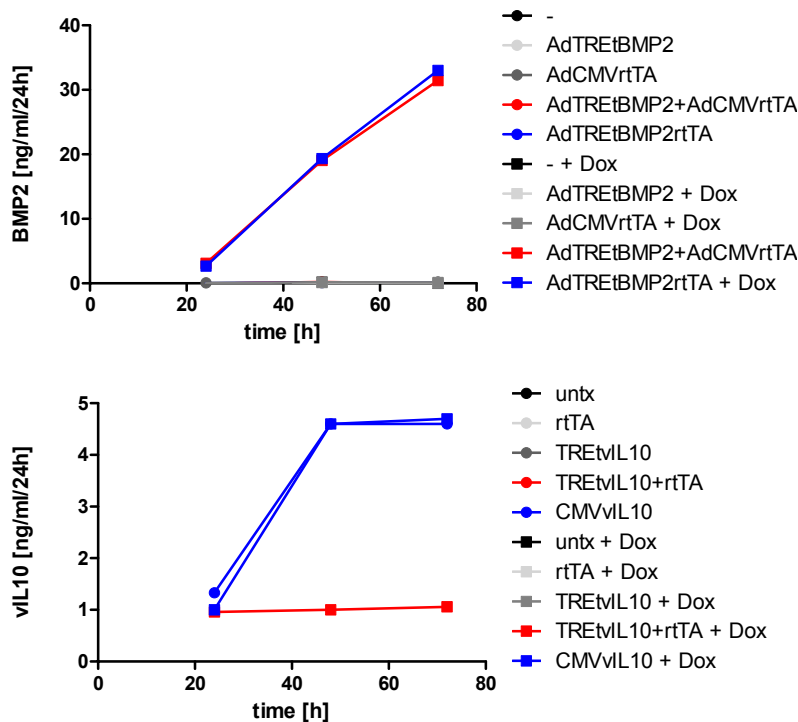


Fig. 1. 1: Quantification of Tet-on inducible expression of BMP-2 (top) and vIL10 (bottom): rMSC were adenovirally infected using a one or two-vector system for BMP-2 and a two-vectors system for regulatable vIL10 expression. Secretion of growth factors into the tissue culture medium were detected by specific ELISAs.

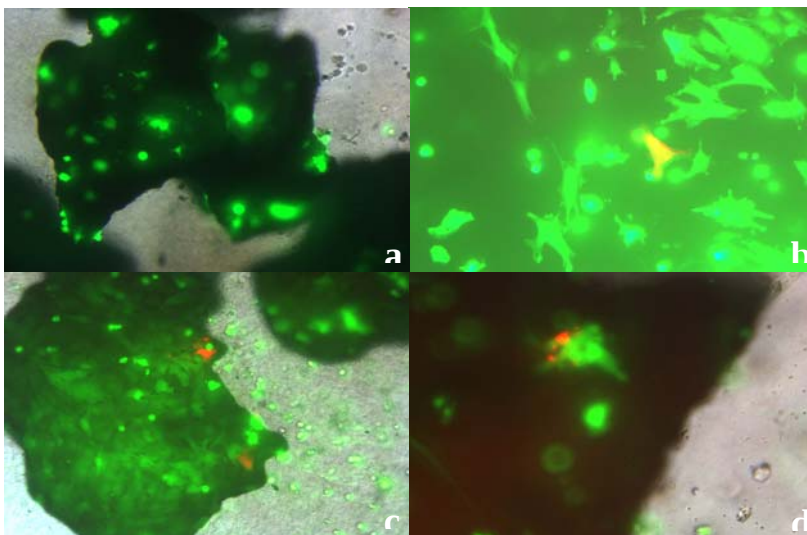


Fig. 1.2: Spatial control of gene expression in 3D assemblies: Fluorescence microscopic images of HA-gel (loaded with AdCMVeGFP) and MBP (loaded with DOGTOR-pCMVsRed2-N1KanR) mixed either with rMSC-cells (a und b) or chondrocytes (c und d) 3 days after colonization. eGFP expressing cells showed successful transduction whereas cells transfected with lipoplexes exhibited a lower transfection efficiency (red fluorescent cells in b-d).

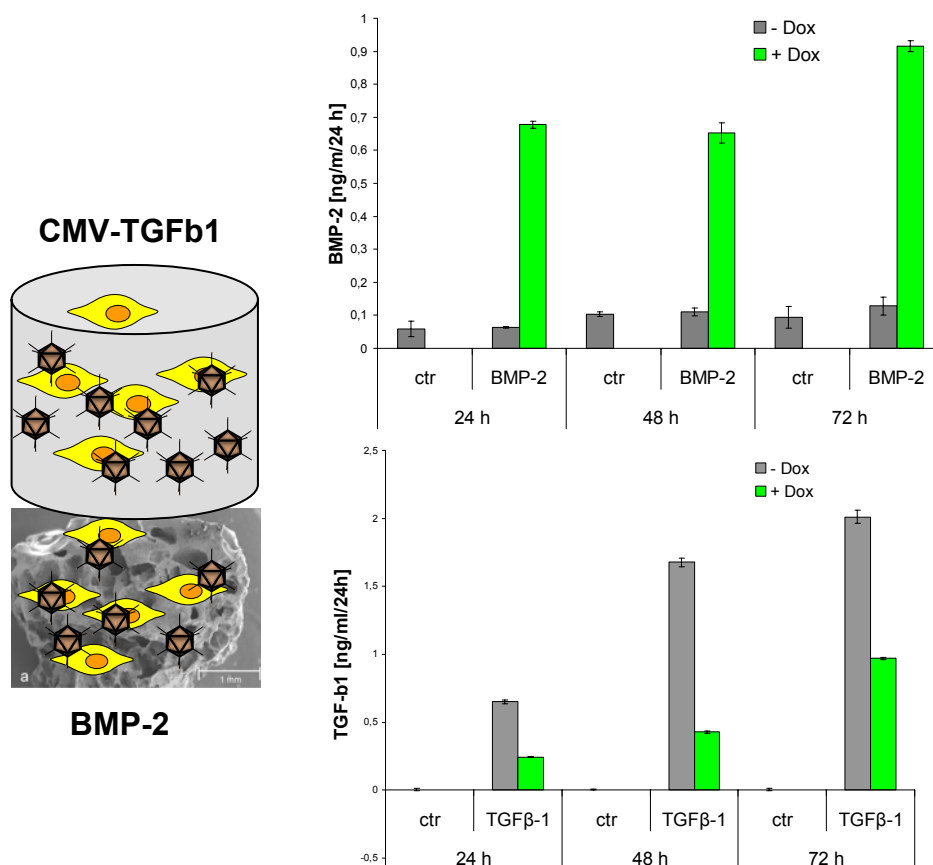


Fig. 1.3: Spatio-temporal control of gene expression. In 3D assemblies using adenoviral vectors (TUM), the temperature sensitive HA gel (ARI) and MBCP+ (BIM/INSERM) and rMSC Dox-mediated induction of BMP-2 (top) expression combined with constitutive expression of TGF-β1 (bottom) was demonstrated.

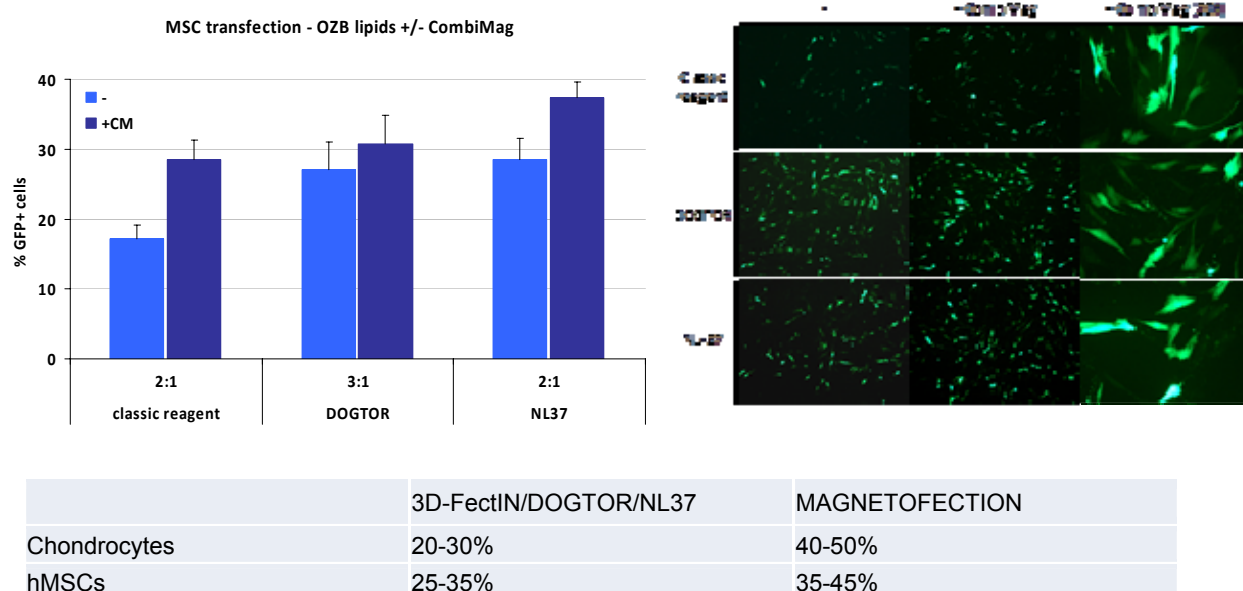


Fig. 1.4: Selection of the best formulation to transfect chondrocytes and hMSC in classical cell culture conditions. DOGTOR and NL-37 induced higher transfection efficiency in terms of number of cells transfected and transgene expression. Moreover, Magnetofection (CombiMag) allowed raising the transfection efficiency of both reagents.

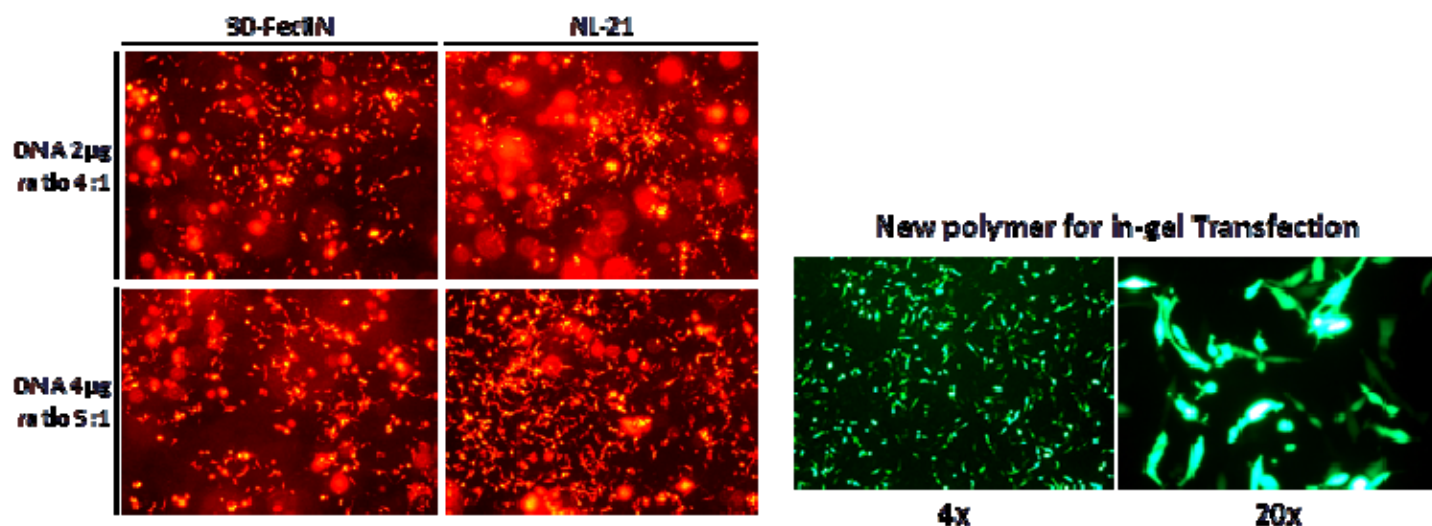


Fig. 1.5: New Lipids and polymer with reduced hydrophobic properties were synthesized. To circumvent the problem of cationic lipids with HA-PNIPAM gels encountered by the partners, OZB has synthesized new lipids polymers with reduced hydrophobic properties.

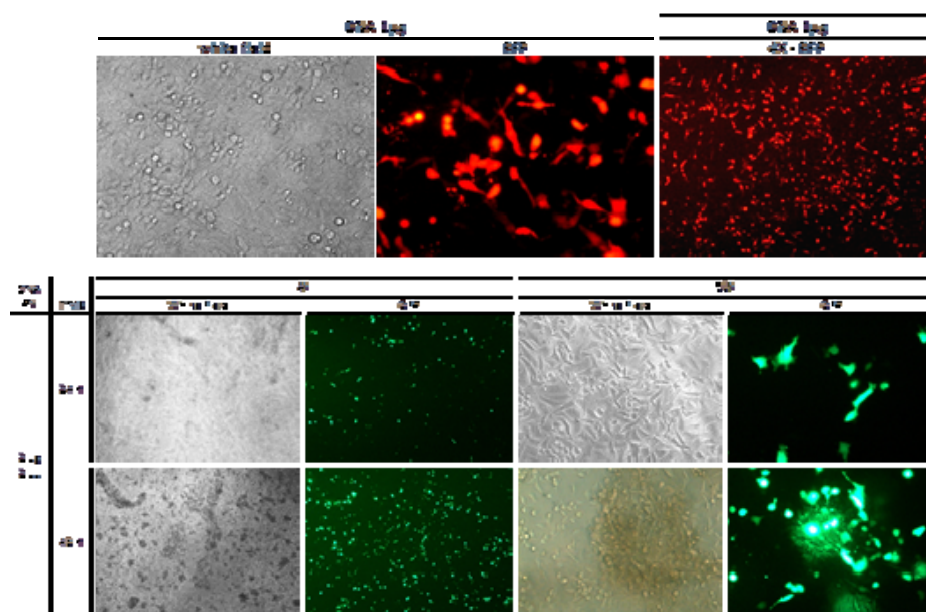


Fig. 1.6: The magnetic nanoparticles from OZB were further optimized for non-viral delivery and assayed on hydrogel. Several formulations were able to transfect cells in atelocollagen hydrogel. One magnetic nanoparticles formulation has transfected cells with high efficiency and lead to rapid gene expression (as soon as 24h) after cell seeding. OZB proceeds with the development of magnetic nanoparticles formulation for optimal performance in 3D culture models.

WP2

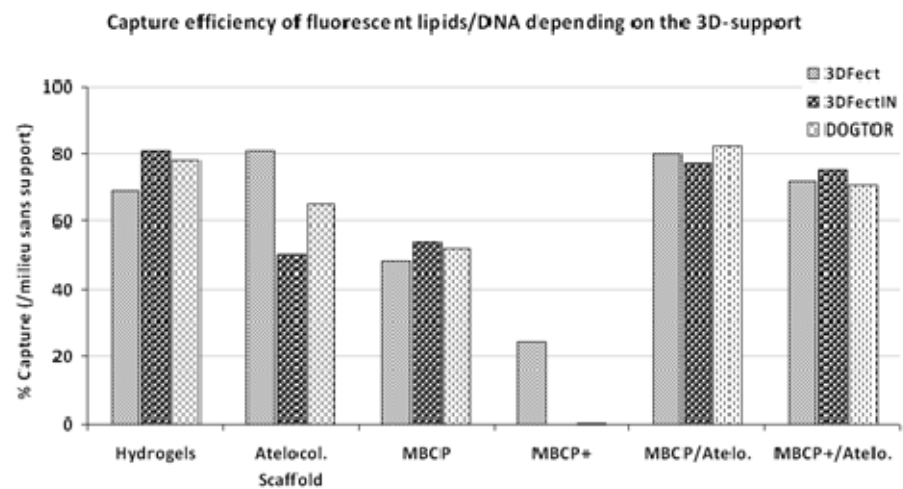


Fig. 2.1: Capture efficiency of fluorescent lipid/DNA on different materials: the retention capacity of the matrices depends on the matrix composition itself and the lipid used for complexes formation.

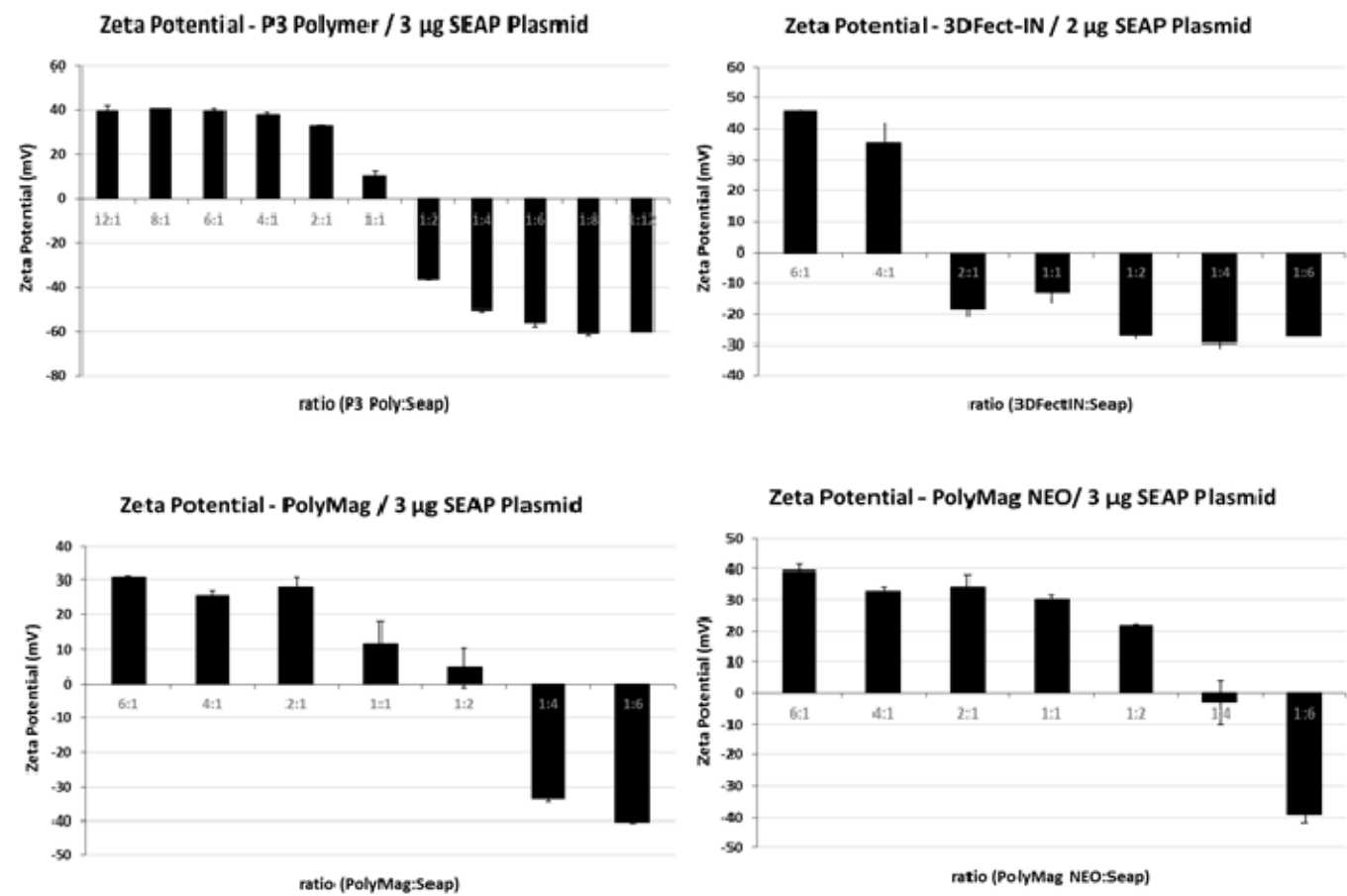
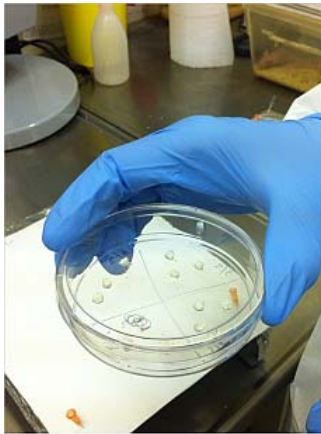


Fig. 2.2: Zeta Potential of DNA/transfection agents at different ratio.



1- Complexes loaded scaffolds preparation



2- Mouse preparation



3- Scaffold insertion under the skin



4- mouse recovery until evaluation of the experiment

Fig. 2.3: in vivo mouse study using assembly modules: Gel containing complexes formed by DOGTOR and DNA were used to prepare matrices of MBCP granules. The modules were then implanted into mice and GFP expression was evaluated after several weeks.

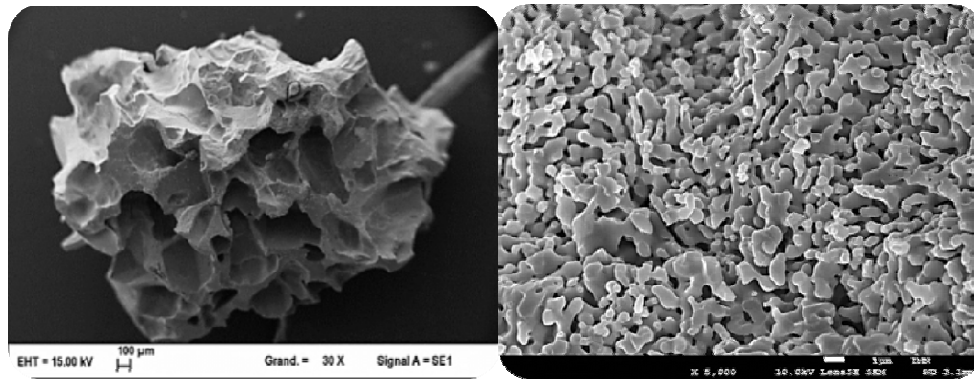


Fig. 3.1: MBCP technology. Macroporosity (left), Microporosity (right)

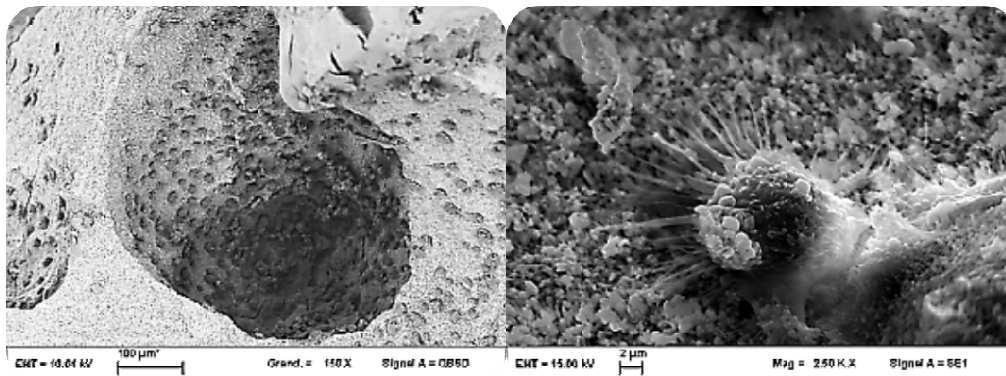


Fig. 3.2: MBCP technology. Cells adhering into macroporosity (left), unique cell spreading at the surface (right)

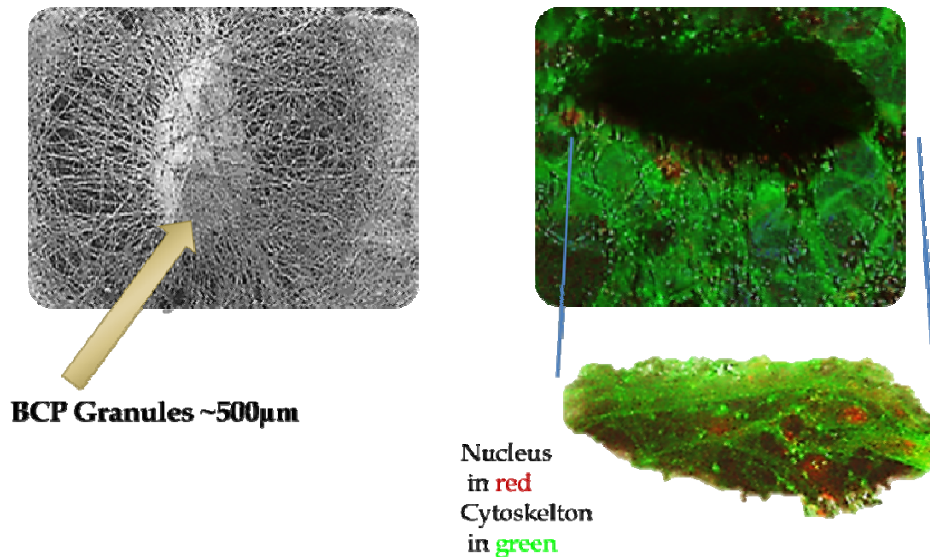


Fig. 3.3: New granules formulation. New granule embedded in fiber based matrix (left), cells proliferating along fibers and inside large internal protective niche concavity of new granules (right)

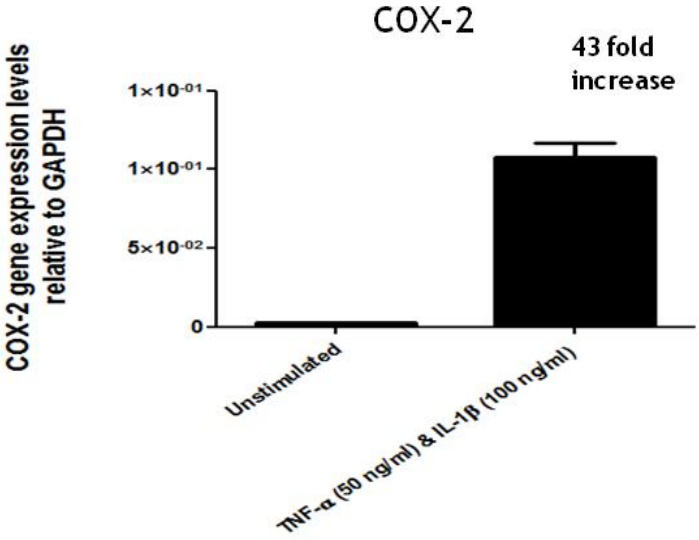


Fig. 4.1: Effect of pro-inflammatory cytokines on COX-2 gene expression. hMSCs were stimulated with 50 ng/ml TNF- α and 100 ng/ml IL-1 β for 24 h. COX-2 gene expression was quantified by PCR. COX-2 expression was clearly elevated in hMSCs following stimulation with this cytokine cocktail.

vIL-10 release following transduction of hMSCs

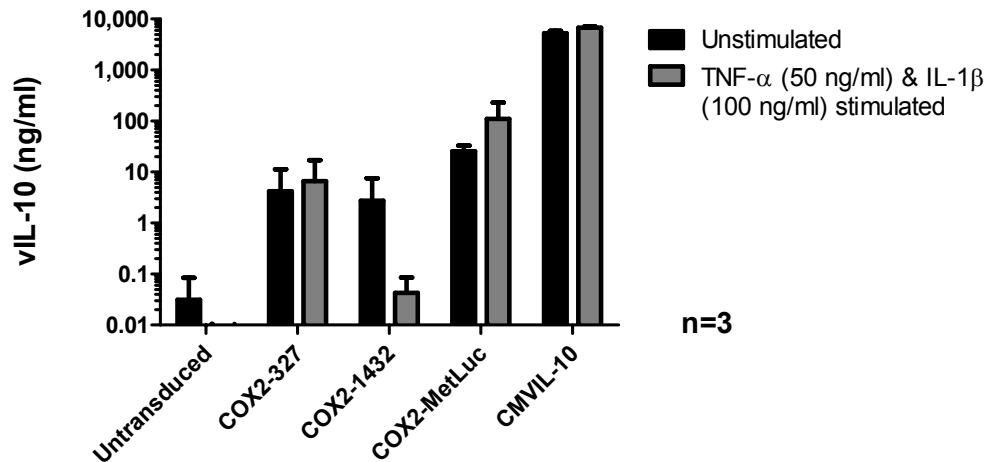


Fig. 4.2: Effect of pro-inflammatory cytokines on the induction of vIL-10 release from hMSCs under the control of the COX-2 promoter.

hMSCs were transduced with COX-2 promoter driven vIL-10 vectors AdCOX2(-327/+59)vIL10, AdCOX2(-1432/+59), AdCOX2 (-1432/+59) and CMVIL-10 at MOI 100. 50 ng/ml TNF- α and 100 ng/ml IL-1 β were added 72 h post transduction. Secreted vIL-10 levels were quantified at 72 h post cytokine stimulation by ELISA. There was a clear induction of vIL-10 in all COX-2 promoter driven samples both with and without cytokine stimulation.

vIL-10 release following transfection of hMSCs using lipofectamine

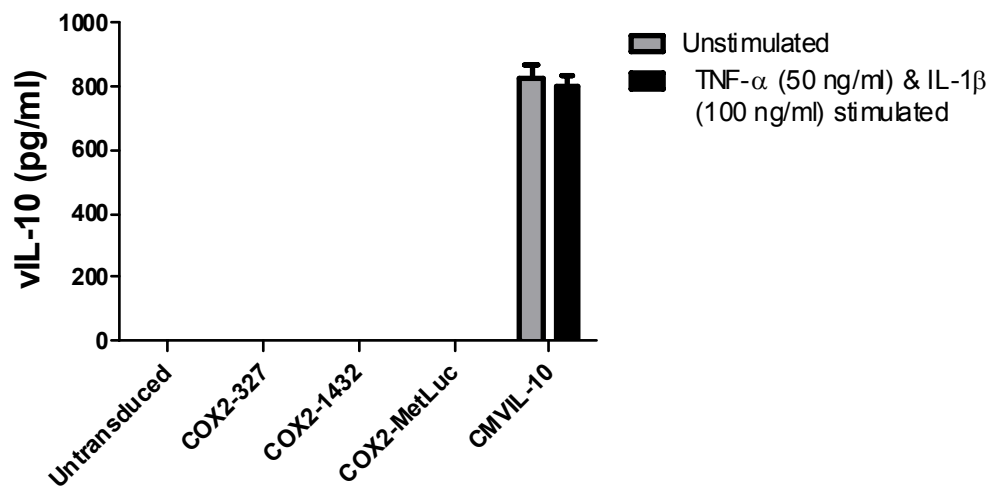


Fig. 4.3: vIL-10 release following transfection of hMSCs using lipofectamine. hMSCs were tranfected with 0.8 μ g of COX2-327, COX2-1432, COX2-MetLuc and CMVIL-10 plasmid DNA. hMSCs were stimulated with pro-inflammatory cytokines at 24 hr post transfection. Cell supernatants were harvested at 24 hr post stimulation. vIL-10 release levels were quantified by ELISA.

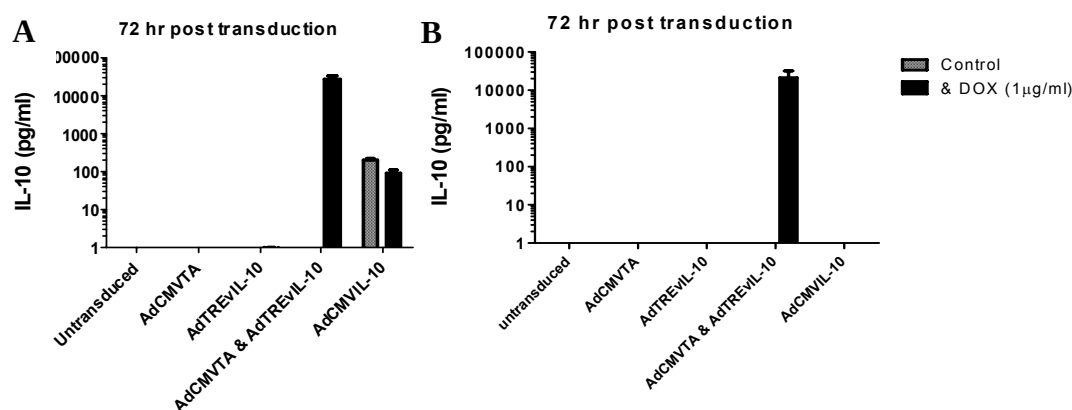


Fig. 4.4: Induction of vIL-10 release following doxycycline stimulation of AdTREvIL-10 and AdCMVTA transduced hMSCs (A) and murine C57BL6 MSCs (B).

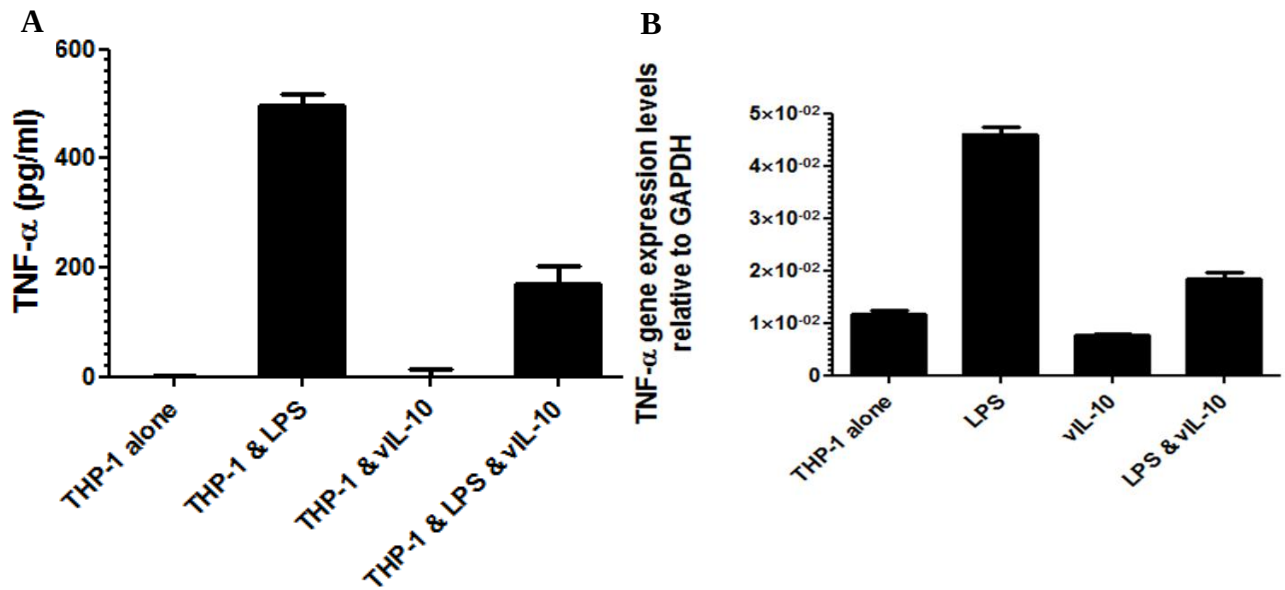


Fig. 4.5: TNF- α release by THP-1 monocytes following stimulation with vIL-10 conditioned media and LPS.

THP-1 monocytes were stimulated with 0.5 ng/ml LPS and 100 ng/ml CMVIL-10 conditioned media, alone or in combination for 48 h. TNF- α release levels were quantified by ELISA (A) and gene expression by PCR (B).

WP5

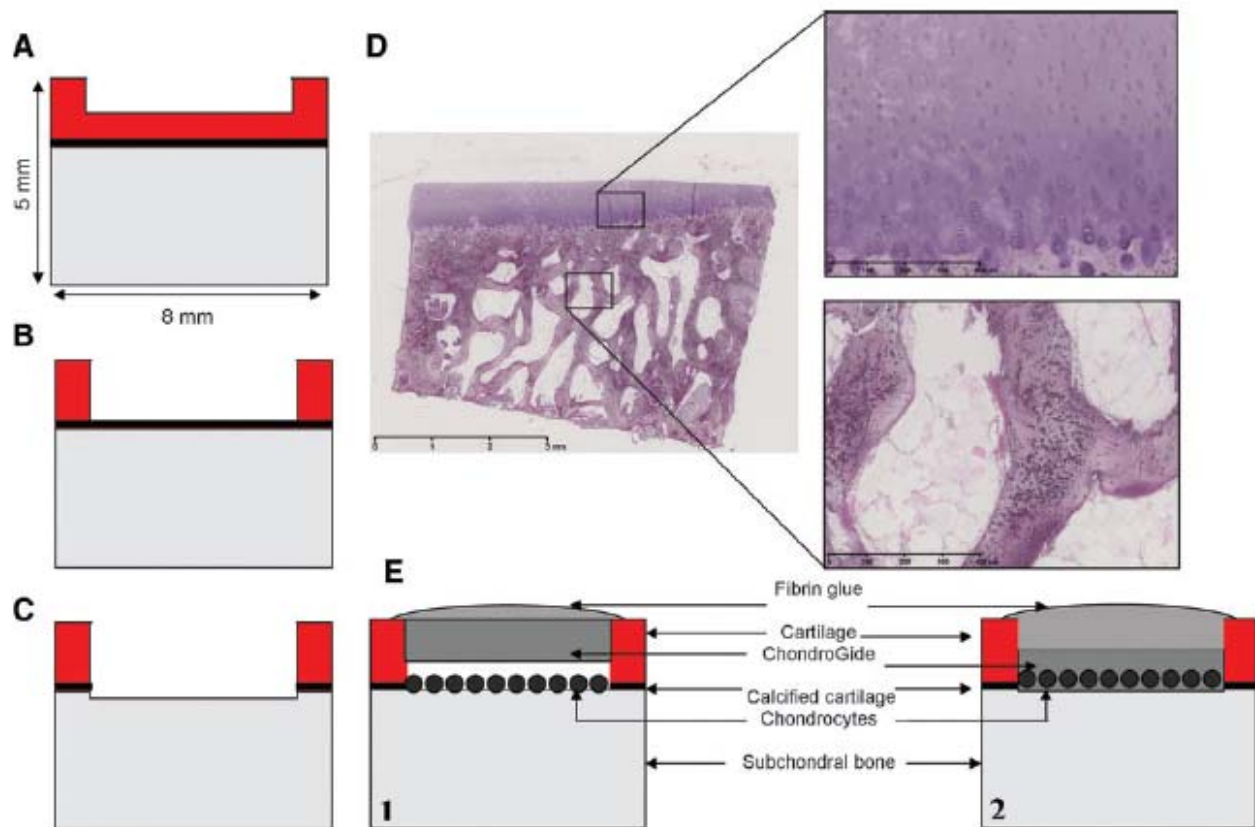


Fig. 5.1 Ex vivo biopsy model. Schematic representation of the ex vivo biopsy model with a chondral defect (a), with a subchondral defect (B), with an osteochondral defect (C), (D) H&E-stained osteochondral biopsy after 28 days of culture; scale bar indicates 3mm. Insets: detail images of cartilage and bone, respectively, scale bars indicate 400 μm. (E) Schematic representation of validation settings of the osteochondral biopsy model

WP6

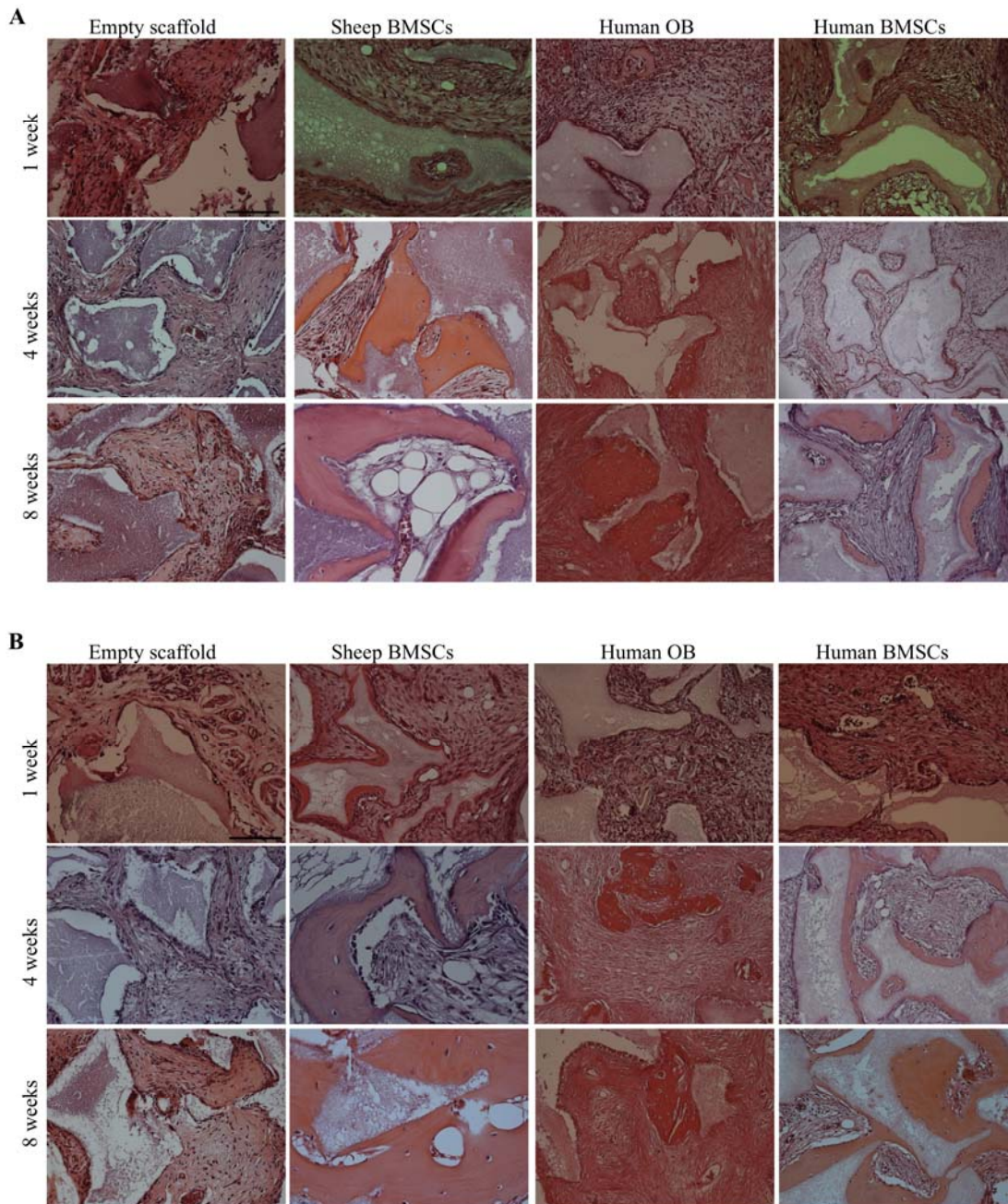


Fig. 6.1 – Bone formation of sheep BMSCs (positive control), human BMSCs and human OB on ceramics of different composition after nu/nu mice implantation. (A) Paraffin histological sections of MBCP A stained with Hematoxylin & Eosin (HE). No osteoconductive properties of this scaffold were observed after implantation without cells proved by the absence of bone formation. A fibrous tissue presenting some parallel orientation was observed (left column of images). Sheep BMSCs and Human OB formed large amounts of bone in 8 weeks explants (2nd and 3rd column). No bone or just a small amount of bone deposition was observed after 1 and 4 weeks of MBC A seeded with human BMSCs (first two pictures of the last column) while 8 weeks explants showed some bone formation (last picture of the column). (B) Histological sections of MBCP⁺ scaffold explants. Scaffold in absence of seeded cells was not able to induce bone deposition by athymic mice recruited cells (1st column of images). Sheep BMSCs seeded within MBCP⁺ scaffold were able after 1 week of implant to deposit new bone (1st image of the second column). Upon 4 and 8 weeks areas of newly bone populated almost the total cellular area of the scaffold (3rd image of the column). Human OB formed new bone areas at 4 and 8 weeks explants (2nd and 3rd images of the

third column). Human BMSC within MBCP + scaffold deposited new bone areas at 4 weeks. These newly formed bone zones were bigger in size at 8 weeks. Hematoxylin & eosin (HE) stains the newly formed bone in orange, the scaffold is shown in smooth green. Scale bar = 100 μ m.

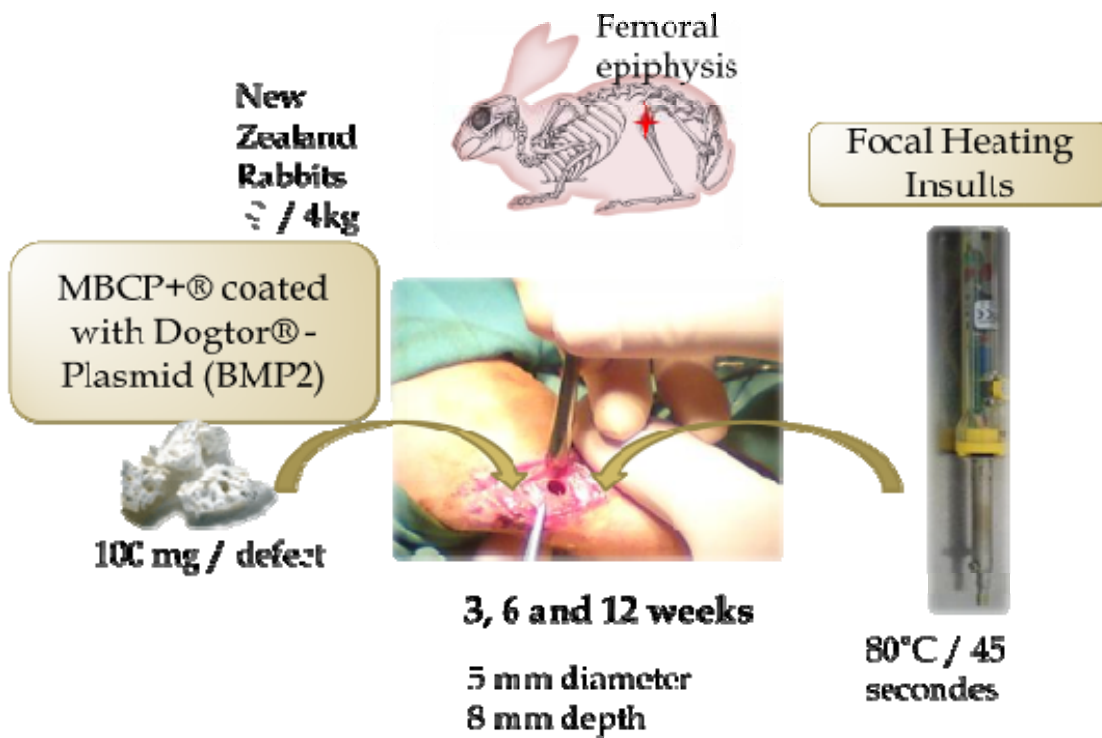


Fig. 6.2: Principle of the in vivo experiment using osteonecrosis model by focal heating insults in rabbit femoral epiphysis

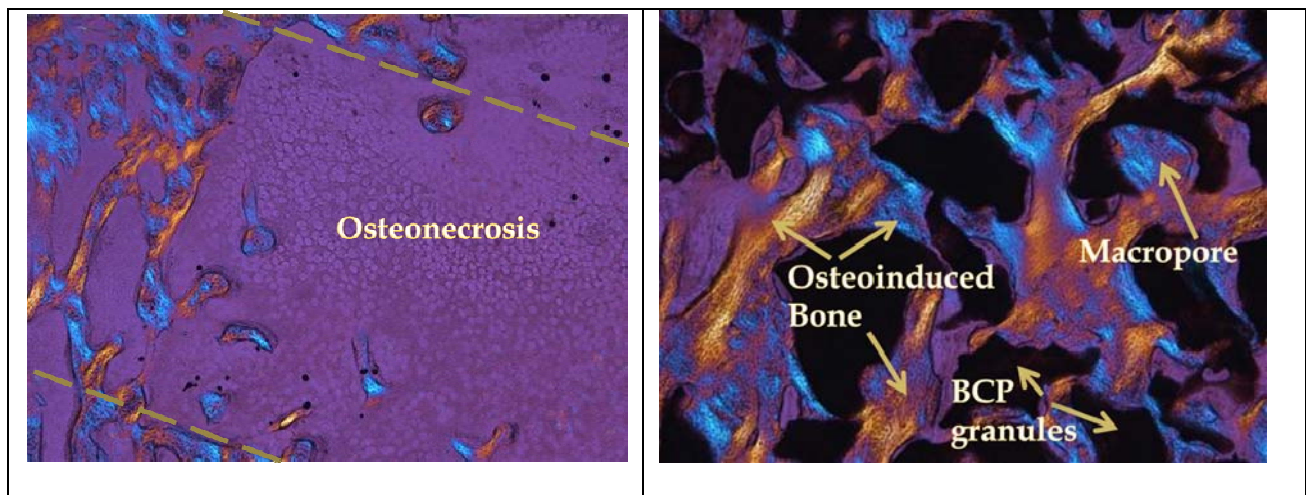


Fig. 6.3: Osteoinductive effect of MBCP+ granules observed after 12 weeks by polarized microscopy on thin layer of explant embedded in acrylic resin. Lamellar mature bone (blue, orange and purple) appears at close contact of ceramics.

WP8



Fig. 8.1 Participants of the German GAMBA citizen panel discuss in small groups



Fig. 8.2 The Swiss GAMBA panel with facilitators Thomas Bänninger and Maren Schüpphaus (centre)