Nanoparticles for regenerative medicine

Marina Pöttler¹, Iwona Cicha¹, Harald Unterweger¹, Christina Janko¹, Ralf P Friedrich¹ & Christoph Alexiou^{*,1}

¹Department of Otorhinolaryngology, Head & Neck Surgery, Section of Experimental Oncology & Nanomedicine (SEON), Else Kröner-Fresenius-Stiftung-Professorship, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Glueckstr. 10a, 91054 Erlangen, Germany

*Author for correspondence: c.alexiou@web.de

First draft submitted: 18 April 2019; Accepted for publication: 20 May 2019; Published online: 2 August 2019

Keywords: cilia restoration • nanoparticles for functional recovery • osteogenesis • remyelination • tissue regeneration

Magnetic-assisted osteogenesis in a 3D collagen model

Evaluation of: Yuan Z, Memarzadeh K, Stephen AS, Allaker RP, Brown RA, Huang J. Development of a 3D collagen model for the *in vitro* evaluation of magnetic-assisted osteogenesis. *Sci. Rep.* 8(1),16270 (2018) – written by Marina Pöttler

Biological behavior of cells can be modified with an induced magnetic field if magnetic nanoparticles are localized within the cells or associated with cell membrane. A high magnetic gradient can lead to a shift of the nanoparticles along the gradient vector, which causes compression and stretch forces on the cell membrane. This phenomenon can be very useful for regenerative medicine, especially for tissues that need mechanical forces as growth- and proliferation-stimuli. Although magnetic stimulation was previously used to induce bone regeneration [1], the underlying mechanisms are not well understood.

To gain more insights in such process, Yuan *et al.* used static magnetic fields in a 3D cell culture model in order to induce the growth of MG-63 human osteosarcoma cells. Magnetic nanoparticles, which were taken up by the cells, served as internal stimulus and the applied static magnetic field was used as an external growth-promoting stimulus. Cells were cultured in a 3D collagen bioreactor for up to 42 days and were analyzed for proliferation, differentiation, mineralization and gene expression. The cells in the scaffold were exposed to the magnetic field alone, the nanoparticles alone, or a combination of both.

Compared with unstimulated controls, cell proliferation was induced in all three treatment groups between day 1 and 14. After 21 days, cell differentiation was significantly increased in cells stimulated with magnetic field alone, and even more in cells exposed to the combination of nanoparticles and magnetic field, whereas cells stimulated with nanoparticles alone did not show an increase in differentiation. A higher level of mineralization was detected in scaffolds exposed to magnetic fields at day 21, but after 42 days no differences in mineralization were seen between the samples, indicating that stimulation with a magnetic field can induce earlier mineralization. Using real time-PCR, the authors investigated the expression of several key genes for osteogenesis including, *Runx2*, osteonectin-encoding *SPARC* gene, *BMP-2* and *BMP-4*. *Runx2* was upregulated using the combination of nanoparticles and magnetic field after 7 and 14 days. Osteonectin-encoding *SPARC* was only upregulated by combined stimuli at day 7, but at day 14, the control group reached the same level. *BMP-2* and *BMP-4* were also only upregulated by the combination of both stimuli at all time points.

The presented biomimetic 3D collagen model allows long-term culture of cells, which are associated with magnetic nanoparticles and can thus respond to magnetic stimulation. Using this tool, the authors demonstrated that the combination of static magnetic field and magnetic nanoparticles can enhance osteogenesis of MG-63 by upregulating the expression of *Runx2*, *SPARC*, *BMP-2* and *BMP-4*. These data indicate the great potential of their model as a platform to study the mechanisms of magnetic stimulation in osteogenesis, which can also be applied to other applications in tissue regeneration research.

Magnetic particles for targeted drug delivery & remote control of primary cilia

Evaluation of: Pala R, Mohieldin AM, Sherpa RT *et al.* Ciliotherapy: remote control of primary cilia movement and function by magnetic nanoparticles. *ACS Nano* 13(3), 3555–3572 (2019) – written by Iwona Cicha and Christoph Alexiou.

Primary cilia, membrane-bound organelles protruding into extracellular space, participate in mechanosensory and chemosensory signaling in most human cell types. Defects in cilia functions or structure (ciliopathies) lead to a wide spectrum of organ specific but also systemic disorders, such as polycystic kidney disease, hypertension, cardiovascular disorders and aberrations of vascular homeostasis [2]. To address this problem, Pala *et al.* have previously designed cilia-targeted nanosystems carrying an experimental drug fenoldopam, which acts by extending cilia length [3]. The particles were based either on metal (Au) or polymer poly(lactic-co-glycolic acid) (PLGA) core and, when tested in a mouse model of hypertension induced by endothelium-specific knockout of polycystin-2-encoding *PKD2* gene, effectively reduced blood pressure, improved heart function and increased nitric oxide bioavailability, as compared with free fenoldopam-treated animals.

Most recently, the authors investigated the possibility of further improving functionality of their nanosystem by using magnetic particles. The resulting ciliotherapeutic construct was based on iron oxide nanoparticles loaded with fenoldopam and targeted to cilia (CT-IO-F) using antibodies directed to dopamine receptor type 5. The specific targeting of primary cilia was confirmed both *in vitro* and *in vivo*. Using CT-IO-F in combination with an external magnetic field, the movement of the nonmotile cilium was induced. Both magnetic and flow-induced cilia movement improved their function as shown by increased calcium signaling and nitric oxide (NO) production both in porcine kidney cell line LLC-PK1 and in isolated primary endothelial cells. Compared with free fenoldopam, intravenously administered CT-IO-F strongly reduced blood pressure in a mouse model of *PKD2* knockout-induced hypertension, which was further decreased in animals receiving nanoparticles in the presence of magnetic field. After 8 weeks of blood pressure studies, heart function was examined *ex vivo*, showing pronounced hypertrophy and functional decline in hypertensive controls. In contrast, CT-IO-F particles improved left ventricular pressure, stroke volume, ejection fraction and overall cardiac output. Importantly, in the animals receiving CT-IO-F in combination with magnetic stimulation applied every 72 h, cardiac function was further improved returning to the levels of wild-type, nonciliopathic controls.

In conclusion, the functional regeneration of primary cilia in a mouse model of hypertension using the developed CT-IO-F was strongly vasculo- and cardioprotective. By efficient targeting of cilia and the resulting increase in calcium signaling leading to NO synthesis, the ciliotherapeutic magnetic nanoparticles prevented the hypertension-induced cardiac hypertrophy in mice. These data suggest that targeted restoration of primary cilia length and motility can represent a promising treatment option in patients with cilia dysfunction-related diseases.

Nanoparticle-based pH buffer prevents the PLGA-induced inflammation

Evaluation of: Lih E, Kum CH, Park W *et al.* Modified magnesium hydroxide nanoparticles inhibit the Inflammatory response to biodegradable poly(lactide-co-glycolide) implants. *ACS Nano* 12(7), 6917–6925 (2018) – written by Harald Unterweger.

Synthetic biodegradable polymers, such as PLGA, have been widely used as coating or matrix material in drug delivery, tissue engineering and regenerative medicine applications. *In vivo*, these systems degrade by hydrolysis of their ester bonds, resulting in acidic degradation byproducts. Yet, these acidic byproducts can react with the surrounding tissue, leading to the induction of (chronic) inflammation, which can cause the subsequent failure of the system [4]. In their work, Lih *et al.* developed a method to incorporate uniformly dispersed $\text{Mg}(\text{OH})_2$ nanoparticles in a PLGA matrix. To achieve this and prevent the particles from aggregation during the incorporation process, their surface was modified in a two-step process with ricinoleic acid and L-lactide, leading to the formation of oligolactide on the particle surface. In contrast to the unmodified nanoparticles (15 wt%), the functionalized $\text{Mg}(\text{OH})_2$ particles incorporated in the PLGA matrix led to a smooth polymer surface with a uniform Mg distribution. Additional advantage of the uniform particle distribution was an increase in tensile strength. In a degradation study, the composite material could maintain the pH of the system closely at neutral pH, while the pure PLGA material caused a significant drop in pH below 3.

In vitro studies with human umbilical vein endothelial cells showed an increase in cell viability for the composite material (90 vs 40%). Additional ELISA experiments with human umbilical vein endothelial cells and the U937 monocytic cell line also demonstrated reduced expression of important inflammation markers, IL-6 and $\text{TNF-}\alpha$, respectively. Two *in vivo* studies were performed to confirm the advantages of the composite material. In the first

study, coronary stent implantation was performed in a porcine model of neointima formation. Compared with the control PLGA stent, the composite stent achieved a better regeneration of the tissue, with significantly lower arterial wall injury, inflammation and fibrin scores, resulting in improved prevention of in-stent restenosis. The second *in vivo* study was performed in a mouse model of partial nephrectomy, aiming at the reconstruction of renal glomerular tissue. One week after implantation, the composite scaffold led to higher cell densities and a more glomerular-like tissue structure compared with pure PLGA scaffolds. In addition, the secretion of inflammatory proteins was also significantly reduced in response to the composite implants.

All in all, these data indicate the great potential of the buffered-PLGA matrix to amend the drawbacks associated with PLGA implants and may facilitate the clinical translation of such systems in the future.

M2 macrophage polarization by IL-4-loaded nanoparticles for functional muscle recovery

Evaluation of: Raimondo TM, Mooney DJ. Functional muscle recovery with nanoparticle-directed M2 macrophage polarization in mice. *Proc. Natl Acad. Sci. USA* 115(42), 10648–10653 (2018) – written by Christina Janko.

Whereas acute inflammation helps to kill invaders, persisting chronic inflammation contributes to the progression of many diseases. During acute infection, circulating monocytes differentiate into macrophages with pro-inflammatory M1 phenotype. Afterward, macrophages switch toward an anti-inflammatory multiple sclerosis (MS) phenotype that supports healing and tissue regeneration [5]. The imbalance of M1 over M2 macrophages can cause excessive chronic inflammation leading to tissue damage or insufficient regeneration, for example, of skeletal muscles after injury [6]. An anti-inflammatory cytokine, IL-4, is explored as potential therapeutic agent in various inflammatory diseases [7]. Of note, IL-4 can induce the switch from M1 to M2 phenotype, however, its short half-life resulting in the need of repeated high dose infusions and systemic side effects limit its *in vivo* use [8].

To deliver IL-4 to the target tissue, Raimondo and Mooney synthesized gold nanoparticles (referred to as PA4), which were partially PEG-stabilized and loaded with IL-4 to the remaining gold surface. PA4, which had hydrodynamic diameter of 30 nm, were stable in medium containing 10% serum and released less than 1% of IL-4 after 7 days. PA4 showed no toxicity toward THP-derived macrophages but upregulated CD206 (M2a marker) to the same extent as soluble IL-4, indicating full bioactivity of nanoparticle-bound IL-4. Contrarily, CD163 (M2c marker) or CCR7 (M1 marker) were not upregulated. PA4-induced M2 polarization of macrophages persisted longer (5 days) than observed with soluble IL-4. Additionally, increasing IL-4 valency on PA4 resulted in higher levels of M2a polarization. The efficacy of PA4 was tested *in vivo* in C57BL/6/J mice with ischemic tibialis anterior muscle. Upon treatment with intramuscular injection of soluble IL-4 or PA4, hematoxylin/eosin staining revealed immune cell infiltration after 3 days, which had disappeared in both study groups after 12 days. Additionally, muscle fiber area was increased. Control mice treated with unloaded nanoparticles did not show immune cell infiltration or muscle fiber gain. At 12 days postinjection, PA4 treated mice showed even higher contraction force and velocity compared with mice receiving soluble IL-4 bolus, unloaded nanoparticles or PBS. Flow cytometry of immune cell infiltrating into the injured tibialis anterior muscles showed a more rapid clearance of CD45⁺ immune cells, monocytes and macrophages in PA4 group compared with PBS-treated controls. Analyzing the macrophage phenotypes, PA4-treated mice showed an increase in CD206⁺ cells and decrease in CD80⁺ cells compared with PBS group, indicating a switch from M1 to M2a phenotype. Interestingly, bolus IL-4 and unloaded nanoparticles reduced M1, but did not promote M2a. To determine if the shift of M1 to M2a played a role in muscle regeneration, macrophages were depleted before ischemic injury using clodronate. With macrophage depletion and PA4 treatment, muscle contraction force and velocity were reduced compared with mice receiving PA4 without macrophage deletion, indicating their central role for muscle regeneration.

Summarizing, the ability of PA4 to direct M2 macrophage polarization may be beneficial not only in the treatment of muscular dystrophies, but also in the treatment of various other inflammatory conditions.

Nanoparticle-based remyelination of the CNS in a rat model

Evaluation of: Youssef AEH, Dief AE, El Azhary NM, Abdelmonsif DA, El-Fetiany OS. LINGO-1 siRNA nanoparticles promote central remyelination in ethidium bromide-induced (EB) demyelination in rats. *J. Physiol. Biochem.* 75(1), 89–99 (2019) – written by Ralf P Friedrich.

Multiple sclerosis (MS) is one of the most common neurological disorders of the CNS. MS is characterized by the loss of the myelin sheath, resulting in demyelinating plaques with variable degrees of inflammation, gliosis and neurodegeneration [9,10]. The treatment of MS is currently limited to drugs with anti-inflammatory or immunomodulatory activity that inhibit inflammation-driven tissue injury [11]. Current developments in MS

therapy aim to stop or reverse the demyelination process itself, for example, by influencing the signaling pathways involved in myelination process. As an example, inhibition of LINGO-1, which belongs to the family of leucine-rich repeat proteins playing key roles in CNS biology, is known to promote remyelination in CNS by stimulating oligodendrocyte precursor cells [12,13].

Addressing this pathway, Yousseff *et al.* produced chitosan nanoparticles loaded with *LINGO-1* siRNA (*LINGO-1* siRNA NPs) and investigated the effect of their intranasal administration in a rat model of EB demyelination. The noninvasive intranasal route enables bypassing of the blood–brain barrier to deliver drugs directly to the CNS [14], whereas the EB model, which triggers an extended demyelination lesion, enables a clear temporal separation between de- and remyelination processes. Various behavioral studies using beam balance test, foot fault test, rotarod test and inverted screen test, showed significantly improved balance and coordination, paw strength, reduced numbers of foot slippage and an increased latency to fall off the rotating rod after treatment with *LINGO-1* siRNA NPs compared with untreated EB animals. Quantitative RT-PCR and western blot analysis revealed a strong increase in myelin basic protein mRNA and protein expression in EB rats treated with *LINGO-1* siRNA NPs compared with the controls. Moreover, *LINGO-1* siRNA NPs protected animals against neural apoptosis, as evidenced by decreased caspase-3 activity, and decreased LINGO-1 mRNA and protein expression in pontine tissue. Finally, histopathological analysis of the pontine tissue in *LINGO-1* siRNA NP-treated EB rats showed a partial protection of the axons from demyelination in the demyelination group and more compact myelin sheaths within the demyelinated lesions of the remyelination group. In agreement with previous reports on the neuroprotective effects of chitosan [15], chitosan NPs without siRNA also increased the mRNA and protein expression of myelin basic protein although their effects were less pronounced as compared with *LINGO-1* siRNA NPs.

Thus, based on behavioral testing, biochemical parameters and histopathological findings, intranasal administration of *LINGO-1* siRNA NPs led to a significant improvement of neurological disturbances in the rat model of EB-induced demyelination. These data suggest that noninvasive intranasal administration of *LINGO-1* siRNA NPs could represent a promising therapy for MS.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

- Huang X, Das R, Patel A, Nguyen TD. Physical stimulations for bone and cartilage regeneration. *Regen. Eng. Transl. Med.* 4(4), 216–237 (2018).
- Luu VZ, Chowdhury B, Al-Omran M, Hess DA, Verma S. Role of endothelial primary cilia as fluid mechanosensors on vascular health. *Atherosclerosis* 275, 196–204 (2018).
- Pala R, Mohieldin AM, Shamloo K *et al.* Personalized nanotherapy by specifically targeting cell organelles to improve vascular hypertension. *Nano Lett.* 19(2), 904–914 (2019).
- Ellis SG, Kereiakes DJ, Metzger DC *et al.* Everolimus-eluting bioresorbable scaffolds for coronary artery disease. *N. Eng. J. Med.* 373(20), 1905–1915 (2015).
- Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol.* 11(11), 723–737 (2011).
- Tonkin J, Temmerman L, Sampson RD *et al.* Monocyte/macrophage-derived IGF-1 orchestrates murine skeletal muscle regeneration and modulates autocrine polarization. *Mol. Ther.* 23(7), 1189–1200 (2015).
- Horsfall AC, Butler DM, Marinova L *et al.* Suppression of collagen-induced arthritis by continuous administration of IL-4. *J. Immunol.* 159(11), 5687–5696 (1997).
- Zidek Z, Anzenbacher P, Kmonickova E. Current status and challenges of cytokine pharmacology. *Br. J. Pharmacol.* 157(3), 342–361 (2009).
- Bishop M, Rumrill PD. Multiple sclerosis: etiology, symptoms, incidence and prevalence, and implications for community living and employment. *Work* 52(4), 725–734 (2015).
- Popescu BF, Pirko I, Lucchinetti CF. Pathology of multiple sclerosis: where do we stand? *Continuum (Minneapolis)* 19(4 Multiple Sclerosis), 901–921 (2013).
- Dobson R, Giovannoni G. Multiple sclerosis – a review. *Eur. J. Neurol.* 26(1), 27–40 (2019).

12. Rudick RA, Mi S, Sandrock AW Jr. LINGO-1 antagonists as therapy for multiple sclerosis: *in vitro* and *in vivo* evidence. *Expert Opin. Biol. Ther.* 8(10), 1561–1570 (2008).
13. Mi S, Pepinsky RB, Cadavid D. Blocking LINGO-1 as a therapy to promote CNS repair: from concept to the clinic. *CNS Drugs* 27(7), 493–503 (2013).
14. Meredith ME, Salameh TS, Banks WA. Intranasal delivery of proteins and peptides in the treatment of neurodegenerative diseases. *AAPS J.* 17(4), 780–787 (2015).
15. Hao C, Wang W, Wang S, Zhang L, Guo Y. An overview of the protective effects of chitosan and acetylated chitosan oligosaccharides against neuronal disorders. *Mar. Drugs* 15(4), pii: E89 (2017).