Nanomedicine for cardiovascular disorders

Iwona Cicha¹ , Harald Unterweger¹, Stefan Lyer¹, Christina Janko¹, Ralf P Friedrich¹, Marina Pöttler¹ & 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.alexio@web.de

First draft submitted: 16 October 2019; Accepted for publication: 4 November 2019; Published online: 26 November 2019

Keywords: aneurysm • atherothrombosis • cardiovascular diseases • metabolic disease • myocardial infarction • stroke

Repair of infarcted myocardium with improved miRNA delivery system

Evaluation of: Yang H, Qin X, Wang H *et al.* An *in vivo* miRNA delivery system for restoring infarcted myocardium. *ACS Nano* 13(9), 9880–9894 (2019) – written by I Cicha.

Cardiovascular disease remains the leading cause of death worldwide, being responsible for 31% of all global deaths [1]. In the EU, there were more than 3 million new cases of ischemic heart disease in 2015 [2]. In the USA, coronary events are expected to occur in about 1,055,000 individuals in 2019, including 720,000 new and 335,000 recurrent coronary events [3]. Coronary atherothrombosis leading to myocardial infarction (MI) requires effective therapeutic intervention to prevent heart failure, but the currently available strategies do not induce cardiac tissue repair.

In their paper, Yang *et al.* address the problem of ineffective delivery of therapeutic miRNA to the infarcted myocardium. To improve the safety and efficacy of *in vivo* delivery, the authors designed polymer-based nanoparticles, composed of a PFBT core (poly[9,9-dioctylfluorene-alt-benzothiadiazole]), which has a good biocompatibility and stable fluorescence that allows accurate tracking of the particles, and a DSPE-PEG (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly[ethylene glycol]) shell, which improves stability and protects the conjugated small interfering RNA from enzymatic degradation. Furthermore, the functional groups of DSPE-PEG were functionalized with TAT peptide, a cell-penetrating peptide derived from the transactivator of transcription (TAT) of human immunodeficiency virus and used to overcome the lipophilic barrier of the cellular membranes to deliver cargo inside the cell. As a therapeutic agent, miR-199a-3p was selected, which is capable of triggering the cell-cycle re-entry of rat and mouse adult/neonatal cardiomyocytes [4] and inducing their proliferation.

The produced nanoparticles, termed miNPs, were characterized in terms of physico-chemical properties and stability. miNPs had a hydrodynamic size of approximately 110 nm and a zeta potential of 22 mV. The cytotoxicity of miNPs was very low in the human embryonic stem cell-derived cardiomyocytes (CM) and endothelial cells (ECs) even at high miRNA loading concentrations (100 nM for CMs, 50 nM for ECs) in comparison with the transfection agent lipofectamine, indicating a negligible risk of damage to human cardiovascular cells. Due to the presence of cell-penetrating peptide, more than 60% of miNPs were internalized into the human cardiovascular cells with extremely low leakage. *In vitro*, miNP activated the cell-cycle re-entry of CMs and ECs and induced the HIF-1/VEGF pathway, which can contribute to improved cardiac function post-MI.

The authors therefore investigated whether *in vivo* delivery of miNP restores cardiac function in a rat model of ischemia/reperfusion (MI model). To improve the local delivery, they employed the miNPs suspended within a shear-thinning hydrogel based on elastin-like protein-hyaluronic acid, which was injected directly into the beating rat hearts. In the hydrogel/miNP group, the contraction of the left ventricle was significantly maintained over 3 months as compared with rats treated with hydrogel only, miNP only or hydrogel/miNP-scramble. CM proliferation in the infarct zone and the capillary growth in the border zone were also enhanced in animals treated with hydrogel/miNP, suggesting that miRNA interference caused heart muscle regeneration and the activation of angiogenesis, leading to improved restoration of cardiac function.

Although the long-term risks and consequences of inducing cardiomyocyte proliferation must be evaluated with caution in future studies, this novel miRNA delivery system shows promising potential and can be easily adapted to other disease entities.

Novel stent-based technique for improved treatment of intracranial aneurysms

Evaluation of: Zhang Y, Wang J, Xiao J *et al.* An electrospun fiber-covered stent with programmable dual drug release for endothelialization acceleration and lumen stenosis prevention. *Acta Biomater.* 94, 295–305 (2019) – written by H Unterweger.

The sudden rupture of an intracranial aneurysm is the main reason of the high mortality rate in hemorrhagic strokes, for example, in aneurysmal subarachnoid hemorrhage [5]. Conventional aneurysm treatment strategies, such as endovascular coil embolism, are associated with high risks of bleeding and low rates of complete occlusion [6]. Especially in complicated cases, such as large or wide-necked aneurysms, the risk is significantly higher and accompanied by an increased recurrence rate.

In their work, Zhang *et al.* developed a stent-based method of aneurysm treatment that minimizes the risks of in-stent-acute thrombosis and long-term stenosis, problems that are usually linked to such a technique. To achieve this, they functionalized their stents with two drugs having different release profiles. On one hand, they incorporated paclitaxel (PTX) into mesoporous silica nanoparticles, which were mixed into an electrospun poly-L-lactic acid fiber membrane. The fibers had a diameter of $1.26 \pm 0.64 \mu\text{m}$ and were evenly distributed in a random arrangement around the stent. The mesoporous silica nanoparticles with incorporated PTX were about 110 nm in hydrodynamic size with a mean pore size of 3.17 nm. *In vitro* studies revealed that this resulted in a sustained PTX release over 63 days without an initial burst release, preventing the over-proliferation of smooth muscle cells. On the other hand, VEGF was bound to the surface of the fibers via dopamine, in order to promote the proliferation of vascular ECs. In contrast to PTX, up to 87% of VEGF was rapidly released within the first 3 days. *In vitro*, the different release profiles of the drugs promoted the growth of ECs and inhibited the smooth muscle cell proliferation.

After successfully demonstrating the *in vitro* potential, the authors examined the effects of these stents in a canine vein pouch aneurysm model. For this purpose, sidewall vein patch aneurysms made in the common carotid artery of 8-month-old beagles were treated with the coated stents and compared with clinically used metal ones. While bare stents showed high rates of prolonged endoleaks, drug-loaded covered stents significantly improved the immediate and mid-term complete aneurysm occlusion rate. Moreover, the introduction of the dual-drug system in the stents with their different release profiles promoted an earlier endothelialization and reduced the lumen restenosis.

Overall, these data indicate the great potential of electrospun fiber-covered stents functionalized with PTX and VEGF for the treatment of intracranial aneurysms.

Nanoparticles cloaked in neural stem cell membranes for delivery of glyburide to stroke

Evaluation of: Ma J, Zhang S, Liu J *et al.* Targeted drug delivery to stroke via chemotactic recruitment of nanoparticles coated with membrane of engineered neural stem cells. *Small* 15(35), 1902011 (2019) – written by S Lyer and C Alexiou.

One of the major challenges in order to prevent brain damage following stroke is the delivery of drugs through the blood–brain barrier (BBB) into the ischemic tissue. An immense problem that can occur after a stroke, especially after a major hemispheric infarct, is the swelling of the brain, which can be both life-threatening and induce a long-term damage to brain tissue [1]. Previous studies strongly suggest that the upregulation of the cell membrane channel SUR1-TRMP4 in all cells of the affected neurovascular unit is one of the reasons for edema formation [7]. After promising preclinical trials with glyburide, which blocks this channel, the first exploratory Phase II study (GAMES-RP) recently showed that glyburide can reduce edema formation and improve clinical outcomes [7,8]. However, one of the commonly accepted problems is that the transport of glyburide through the BBB is poor. Therefore, Ma *et al.* exploited the capability of neuronal stem cells (NSC) engineered to overexpress CXCR4 to conquer the brain tissue via the BBB by interacting with SDF-1. The levels of this stromal cell-derived factor are significantly elevated in ischemic regions 24 h after infarction in the brain. Using the genetically modified NSCs, the authors demonstrated their selective accumulation in the ischemic regions in a mouse model of middle cerebral artery occlusion-induced ischemia [3]. Consequently, the authors aimed to prove that this enhanced trafficking into ischemic brain tissue can be utilized as a nanoparticle drug delivery system by coating glyburide-loaded PLGA-nanoparticles (NP) with membranes of NSCs. After the middle cerebral artery occlusion surgery, they administered

animals with glyburide-loaded NPs coated with membranes from CXCR4 overexpressing NSCs (Gly-CMNP) or with the membranes of normal NSCs (Gly-MNP), drug-loaded NPs without NSC-membrane coating or free glyburide and compared the effects of different treatments on the survival and neurological damage. The results showed significantly improved survival, reduced infarct volumes (58%) and improved neurological scores for animals treated with Gly-CMNP. In other treatment groups, including Gly-MNP, glyburide alone or loaded on NPs without membrane coating, an improvement was also noted when compared with the untreated control, but this effect was not as pronounced as in Gly-CMNP-treated animals.

In summary, the authors showed that the application of Gly-CMNPs improves the delivery of glyburide into ischemic brain tissue through the BBB and that this had a significant positive effect on the outcome in the mouse model. These findings may suggest a high potential for clinical translation of their approach. Furthermore, the developed CMNPs could serve as a versatile platform for drug delivery through the BBB into diseased brain tissue.

Liver delivery of berberine micelles to ameliorate cardio-metabolic diseases

Evaluation of: Guo HH, Feng CL, Zhang WX *et al.* Liver-targeting nanotechnology facilitates berberine to ameliorate cardio-metabolic diseases. *Nat. Commun.* 10, 1981 (2019) – written by C Janko.

Cardiovascular and metabolic diseases are the number one cause of premature death worldwide [1]. As alternative to statins, a botanic compound berberine (BBR) serves as an approved nutraceutical substance for the treatment of hyperlipidemia [9]. Despite lowering plasma lipids effectively, it suffers from poor bioavailability and high doses are necessary, which can cause adverse effects [10]. To increase its accumulation in the liver, the target organ for BBR, Guo *et al.* developed a drug delivery system consisting of a D- α -tocopheryl hydrophobic core and an on-site detachable cross-linked polyethylene glycol-thiol shell (CTA-Mic). The BBR-loaded micelles (BBR-CTA-Mic) had a spherical morphology with 54.66 nm particle size, -16.1 mV zeta potential and were stable in simulated physiological fluids. In serum and simulated gastric and intestinal fluids, the BBR-CTA-Mics only slowly released the drug, but had a burst release upon exposure to 20% hepatic homogenate, possibly caused by glutathione-mediated cleavage of the disulfide bond on the outer shell, together with the amide enzyme-induced collapse of succinamide in the inner core.

Studies in Caco-2 cell lines showed that encapsulation in micelles increased the transepithelial effect of BBR by diminishing the efflux by the P-gp membrane pump. Following intestinal absorption, the liver is the first organ for BBR to accumulate in after transport via the portal vein. In HepG2 cells, more intracellular BBR was observed when applied as BBR-CTA-Mic, in contrast to BBR alone or BBR plus CTA-Mic. Concerning biodistribution *in vivo*, after gavage injection in B57BL/6J mice, BBR-CTA-Mic not only reached much higher maximum concentrations, but also showed longer retention times in the liver compared with BBR alone. In particular BBR-CTA-Mic enhanced the expression of energy-metabolism related genes, mRNAs and proteins (p-AMPK, InsR, LDLR), both in HepG2 cells, as well as in livers of mice treated with BBR formulations. In mice with a high-fat diet (HFD), BBR-CTA-Mic significantly reduced the levels of plasma and hepatic triglycerides, cholesterol, LDL-c and blood glucose, as compared with the untreated group. Free BBR also reduced lipids and glucose, but with lower efficacy. In line with this, after 16 weeks medication, the body weights of obese mice had been reduced by BBR-CTA-Mic down to healthy levels. Also, their adipocyte size, mesentery and liver weight, epididymal fat, plasma ALT levels and fatty liver had normalized. In HFD-mice, TNF- α , IL-1 β , IFN- γ and IL-6 plasma levels were substantially increased and treatment with BBR-CFA-Mic greatly inhibited these elevations. A mild decline was also observed with free BBR. IL-6 and TNF- α , being important for atherosclerosis, showed downregulation by BBR-CFA-Mic in liver and fat tissue, down to the levels of healthy mice and atherosclerosis formation in the aorta was accordingly reduced. Concerning long-term safety, no obvious histological differences were found in major organs between the treated and untreated mice, nor in their plasma levels of ALT, AST creatinine and blood urea nitrogen after 4 months.

In conclusion, CTA-Mic were successfully applied to target BBR to the liver, ameliorating metabolic disorders and atherosclerosis in HFD-fed mice. BBR-CTA-Mic might thus represent a promising drug delivery system for treating cardiovascular and metabolic diseases.

Thrombus-specific aspirin particles for the directed therapy of vascular diseases

Evaluation of: Lee J, Jeong L, Jung E *et al.* Thrombus targeting aspirin particles for near infrared imaging and on-demand therapy of thrombotic vascular diseases. *J. Control. Rel.* 304, 164–172 (2019) – written by RP Friedrich.

Atherothrombotic events, resulting from a sudden atherosclerotic plaque disruption or thrombus formation, are the most common cause of death and the leading cause of disability in adults [11]. Early recognition and treatment of the life-threatening clots that obstruct blood vessels supplying vital organs, is essential to reduce mortality and minimize the massive health deterioration after stroke and MI.

In patients with acute ischemic stroke, intravenous thrombolysis using recombinant tissue plasminogen activator is the treatment of choice [12]. However, this is related to a high risk of thrombolysis-induced intracerebral hemorrhagic complications and the outcome is poor when occlusion occurs in large arteries. Hence, there is an urgent need for a safe and targeted therapeutic treatment to dissolve the occlusive clots composed of blood cells, fibrin and activated platelets.

Lee *et al.* described a promising approach using thrombus-targeting aspirin polyconjugate particles (T-APP) as an intravenously injectable agent, which can rapidly target thrombosed vessels and prevent the growth of thrombi. The particles were composed of a copolymer, poly(HEMA-co-MMA), which was covalently conjugated via a degradable linker with ethyl salicylate (ESA), the major active ingredient of aspirin characterized by a strong antiplatelet activity [13]. Additional functionality was provided by the degradable peroxalate ester linker, which is rapidly oxidized by H_2O_2 , thus scavenging H_2O_2 molecules responsible for platelet activation, endothelial dysfunction and the expression of pro-inflammatory proteins [14,15]. The particles were further functionalized with a fibrin-specific pentapeptide, Gly-Pro-Arg-Pro-Pro (GPRPP), acting as a thrombus-targeting ligand and with IR780, as a probe for near-infrared imaging [16].

Characterization using dynamic light scattering and scanning electron microscopy revealed spherical particles with hydrodynamic diameters ranging from 300–400 nm (nontargeted APP) and 500–600 nm (targeted T-APP). H_2O_2 -dependent release experiments showed that APP undergo H_2O_2 -triggered peroxalate ester oxidation, leading to H_2O_2 scavenging and ESA release. *In vitro* experiments with APP using endothelial cells and macrophages clearly showed a concentration-dependent reduction of H_2O_2 -induced intracellular ROS levels and toxicity. Moreover, APP exhibited strong anti-inflammatory effects by suppressing the upregulation of TNF- α in LPS-stimulated cells and sCD40L in activated platelets. Targeting experiments with T-APP confirmed its strong ability to specifically bind fibrin-rich clots, as compared with APP.

In a mouse model of tail bleeding, T-APP-treated animals had a significantly increased bleeding time and bleeding volume compared with the control group, demonstrating the clot-targeting and anti-platelet activity of T-APP. Moreover, in a mouse model of $FeCl_3$ -induced carotid arterial thrombosis, intravenously injected T-APP rapidly accumulated at the site of thrombus formation. $FeCl_3$ -induced TNF- α and sCD40L upregulation was significantly reduced by free ESA and even more efficiently by T-APP, indicating an important role of the fibrin-targeting peptide. Accordingly, histological staining confirmed stronger antithrombotic activity and reduced ROS levels after T-APP treatment compared with free ESA treatment. Finally, long-time administration of T-APP demonstrated a good biocompatibility and no obvious toxicity in mice.

In conclusion, the authors developed multifunctional targeted nanoparticles that can scavenge H_2O_2 , inhibit the expression of TNF- α and sCD40L and exert the antithrombotic activity of ESA.

Vascular grafts for local regulation of inflammation

Evaluation of: Tan RP, Chan AHP, Wei S *et al.* Bioactive materials facilitating targeted local modulation of inflammation. *JACC Basic Transl. Sci.* 4(1), 56–71 (2019) – written by M Pöttler.

Macrophages are able to modify their behavior and accomplish specific functions adapting to various disease processes. Switching from the M1 (pro-inflammatory) to the M2 (anti-inflammatory) state regulates inflammatory cytokines to either propagate or stop innate inflammation [17]. Atherosclerosis and other types of cardiovascular disorders are highly inflammatory diseases and are known to benefit from anti-inflammatory drugs [18]. However, a systemic drug administration against, for example, IL-1 beta, can lead to extreme risk of fatal infection and sepsis.

The engineered vascular grafts are prone to foreign body immune responses, which affect the long-term patency of such devices. Therefore, using a local anti-inflammatory surface coating is expected to overcome this problem. IL-4, known to induce the macrophage phenotype switch from M1 to M2 was chosen by Tan *et al.* to be mobilized on the surface of electrospun polycaprolactone (PCL) nanofibrous scaffold, which shall be used for production of vascular prostheses. Binding of IL-4 to PCL fibers was done without a linker, but by the plasma immersion ion implantation surface treatment, which facilitates rapid covalent attachment of biomolecules while also maintaining their bioactivity. The bioactive material surface (IL-4 surface) and the controls, including polycaprolactone (PCL)

alone, PLC plus IL-4 (passive IL-4), or PLC treated with plasma immersion ion implantation, were generated and characterized.

The authors first analyzed how a macrophage cell line reacts to the bioactive IL-4 surface *in vitro*, by incubation of RAW264.7 cells with respective test materials. Cell phenotype was verified by the analysis of M2 genes, including *arginase-1*, *CD163*, *CD206* or *IL-10*. Only bioactive IL-4 surface was able to induce expression of all four markers.

Next, the produced scaffolds were tested in mouse models for their inflammatory response. Two *in vivo* studies were performed, a subcutaneous implantation and a carotid graft implant, to examine the possible switch of the macrophage type.

Subcutaneous implantation in mice revealed a reduced inflammatory cytokine expression and fibrous encapsulation on the bioactive IL-4 surfaces, compared with control IL-4 surfaces, as well as a higher M2 to M1 macrophage polarization. To demonstrate that the bioactive IL-4 grafts reduce local inflammation and neointimal hyperplasia, the authors used small diameter vascular grafts with the bioactive IL-4 coating on the luminal and adventitial surfaces. In the mouse carotid interposition grafting model, they showed that bioactive IL-4 grafts significantly reduced total macrophage presence, with more remaining macrophages of M2 phenotype. ELISA and immunohistochemical data further verified these results. Although the study limitations include the use of small animal models and the healthy phenotype, the authors successfully demonstrate that their biomaterial used as a vascular grafts, reduced macrophage burden and promotes their M2 polarization. Additionally, a positive regulation of the local cytokine expression, lessening of foreign body reaction and the reduction of neointimal hyperplasia could answer the need of the long-term graft patency by controlling local inflammatory response. Future studies in larger animals and a mouse model of atherosclerosis would certainly give a more specific and realistic evaluation of the benefits of an IL-4 bioactive graft material.

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

1. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 388(10053), 1459–1544 (2016).
2. EU Eurostat: cardiovascular diseases statistics (2018). https://ec.europa.eu/eurostat/statistics-explained/index.php/Cardiovascular_diseases_statistics
3. Benjamin EJ, Muntner P, Alonso A *et al*. Heart disease and stroke statistics-2019 update: a report from the American Heart Association. *Circulation* 139(10), e56–e528 (2019).
4. Eulalio A, Mano M, Dal Ferro M *et al*. Functional screening identifies miRNAs inducing cardiac regeneration. *Nature* 492(7429), 376–381 (2012).
5. Ellegala DB, Day AL. Ruptured cerebral aneurysms. *N. Eng. J. Med.* 352(2), 121–124 (2005).
6. Chalouhi N, Jabbour P, Singhal S *et al*. Stent-assisted coiling of intracranial aneurysms: predictors of complications, recanalization, and outcome in 508 cases. *Stroke* 44(5), 1348–1353 (2013).
7. King ZA, Sheth KN, Kimberly WT, Simard JM. Profile of intravenous glyburide for the prevention of cerebral edema following large hemispheric infarction: evidence to date. *Drug Des. Devel. Ther.* 12, 2539–2552 (2018).
8. Sheth KN, Petersen NH, Cheung K *et al*. Long-term outcomes in patients aged ≤70 years with intravenous glyburide from the Phase II GAMES-RP study of large hemispheric infarction: an exploratory analysis. *Stroke* 49(6), 1457–1463 (2018).
9. Kong W, Wei J, Abidi P *et al*. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat. Med.* 10(12), 1344–1351 (2004).
10. Yan HM, Xia MF, Wang Y *et al*. Efficacy of berberine in patients with non-alcoholic fatty liver disease. *PLoS ONE* 10(8), e0134172 (2015).
11. Jackson SP. Arterial thrombosis—insidious, unpredictable and deadly. *Nat. Med.* 17(11), 1423–1436 (2011).
12. Chapman SN, Mehndiratta P, Johansen MC, McMurry TL, Johnston KC, Southerland AM. Current perspectives on the use of intravenous recombinant tissue plasminogen activator (tPA) for treatment of acute ischemic stroke. *Vasc. Health Risk Manag.* 10, 75–87 (2014).

13. Warkentin TE. Aspirin for dual prevention of venous and arterial thrombosis. *N. Eng. J. Med.* 367(21), 2039–2041 (2012).
14. Leopold JA, Loscalzo J. Oxidative risk for atherothrombotic cardiovascular disease. *Free Radic. Biol. Med.* 47(12), 1673–1706 (2009).
15. Kang C, Cho W, Park M *et al.* H₂O₂-triggered bubble generating antioxidant polymeric nanoparticles as ischemia/reperfusion targeted nanotheranostics. *Biomaterials* 85, 195–203 (2016).
16. Aruva MR, Daviau J, Sharma SS, Thakur ML. Imaging thromboembolism with fibrin-avid 99mTc-peptide: evaluation in swine. *J. Nucl. Med.* 47(1), 155–162 (2006).
17. Atri C, Guerfali FZ, Laouini D. Role of human macrophage polarization in inflammation during infectious diseases. *Int. J. Mol. Sci.* 19(6), 1801 (2018).
18. Fava C, Montagnana M. Atherosclerosis is an inflammatory disease which lacks a common anti-inflammatory therapy: how human genetics can help to this issue. a narrative review. *Front. Pharmacol.* 9, 55 (2018).