Biodegradable Nanoparticles for Specific Drug Transport

Karin Danz, Hagen von Briesen, and Sylvia Wagner

Abstract Biodegradable nanoparticles are highly versatile and adaptable delivery systems for pharmaceutical drugs. The nanoparticles can be targeted to specific cell types and tissues, modified to overcome biological barriers the contained drugs cannot overcome on their own and at the same time mask the undesirable side effects of pharmacological substances. The nanoparticle characteristics can be tailored to specific targets by modifications such as the addition of antibodies to the particle surface or coating them in substances altering their circulation behaviour. Different approaches and examples for targeted drug delivery with biodegradable nanoparticles are discussed, as well as model systems for the advanced evaluation of the used formulations shown.

Keywords Biodegradable nanoparticles · Specific drug targeting · Overcome biological barriers · Blood-brain barrier

Abbreviations

ApoE	Apolipoprotein E
BBB	Blood-brain barrier
EGFR	Epidermal growth factor receptor
EPR	Enhanced Permeability and Retention
EMA	European Medicines Agency
FDA	Food and Drug Administration
HSA	Human serum albumin
HER2	Human epidermal growth factor receptor-2
PLA	Polylactic acid
PLGA	Poly(lactic-co-glycolic) acid

K. Danz · H. von Briesen · S. Wagner (✉)
Department Bioprocessing and Bioanalytics, Fraunhofer Institute for Biomedical Engineering, Joseph-von-Fraunhofer-Weg 1, 66280 Sulzbach, Germany
e-mail: sylvia.wagner@ibmt.fraunhofer.de

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PVA Polyvinyl alcohol
TER Transendothelial electrical resistance

1 Introduction

The targeted delivery of drugs within the human organism is an ongoing challenge in pharmaceutical circles. To this day, the administration of drugs by oral or intravenous means leads to a nearly uniform distribution within the body once initial absorption e.g. through the gastrointestinal tract by oral delivery has occurred. This is followed by enrichment in tissues capable of metabolising or storing the substances, specifically liver, kidney and spleen. The only exception in this systemic distribution in many cases is the brain environment. Here, the blood-brain barrier (see Sect. 4.2) due to its highly selective character prevents more than 98% of all pharmaceutical drugs from crossing into the brain environment (Pardridge 2005). The general systemic distribution provides an advantage when tackling systemic illnesses but, in many cases, a targeted approach would be preferable. Modern drugs still carry the potential for both unwanted side effects of a single drug or problematic drug interactions in case of application of multiple substances. The possibility to deliver a drug only to the tissue or organ of interest has been a research target for decades. One of the most promising options to achieve this goal are nanoparticulate delivery systems. Since their first descriptions in the 1970s, nanoparticles have been investigated for a number of potential applications, not limited to targeted drug delivery (Birrenbach and Speiser 1976; Kreuter and Speiser 1976; Scheffel et al. 1972). The use in diagnostic context for example is of particular interest as well. Here, metallic nanoparticles specifically have proven useful (Mody et al. 2010). Yet, for drug delivery approaches, especially in the case of long-term or continuous medication, the nanoparticle systems need to be biocompatible to such a degree as to not build up over time of administration or instigate an immune response for clearing (Mirabello et al. 2015). A base of biodegradable polymers, that can be degraded and metabolised within existing cellular metabolic pathways, have been the solution of choice for some time.

2 Nanoparticulate Drug Delivery Systems

For drug delivery approaches, nanoparticles are classified as structures with a mean size of 1 to 1000 nm. While a colloidal shape is most common, other shapes including cubic or rod-like are possible (Saraiva et al. 2016). The base material, as mentioned above, is usually comprised of biodegradable polymers. This includes both natural macromolecules like human serum albumin (HSA), gelatine, chitosan or alginate, as well as synthetic polymer blocks like polylactic acid (PLA), poly(lactic-co-glycolic) acid (PLGA), polyacrylic acid and its derivatives and others (Kumari et al. 2010).

The resulting particles exhibit a natural electric charge, that can range from positive to negative with varying degrees of strength. The drugs are either trapped within the particle matrix itself or bound to the particles surface. Additionally, surface modifications are possible to achieve specific characteristics or target certain cells types, organs or tissues (Fig. 1).

The nanoparticle production itself is largely dependent on the base material. Both chemical polymerisation reactions as well as dispersion-based methods have been described (Kreuter 1983). Chemical polymerisation reactions establish crosslinks between the polymer blocks. Controlling this chemical reaction provides a certain degree of adjustability concerning the appearance and characteristics of the produced nanoparticles. With dispersion methods, the polymer blocks are allowed to form nucleation cores and grow within a solution without establishing chemical links

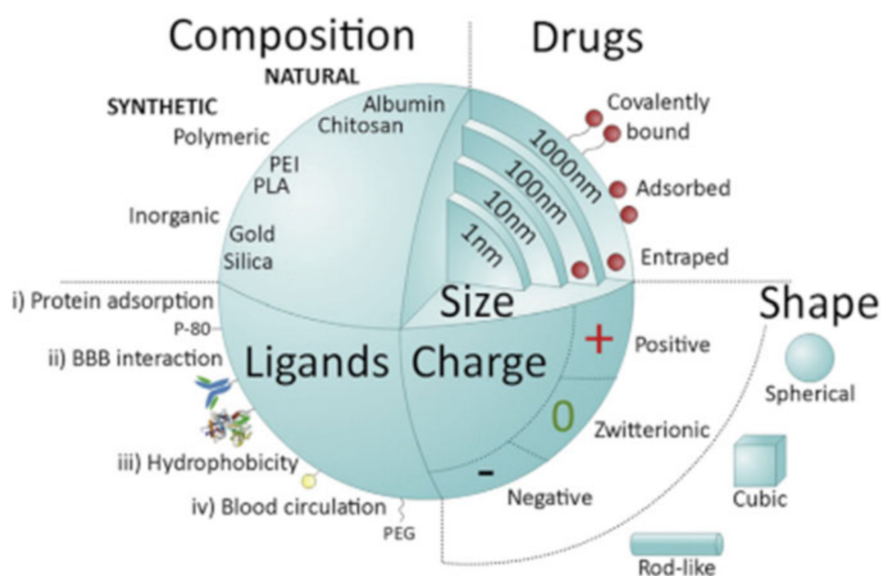

Fig. 1 Key nanoparticle characteristics influencing circulation behaviour and cellular targeting. Nanoparticles can be comprised of natural biopolymers, like albumin, chitosan or gelatin, or of synthetically produced building blocks, such as synthetic polymers, including polylactic acid (PLA), poly(lactic-co-glycolic) acid (PLGA) and poly(ethylenimine) (PEI), or inorganic substances, usually metals (Gold, Silica, Iron). Nanoparticle sizes can range from 1 to 1000 nm and the transported drugs can be covalently bound or adsorbed to the particle surface, as well as entrapped within the particle structure. The shapes of nanoparticles vary, from spherical to cubic, rod-like or tubular, any number of configurations are possible. Nanoparticles can also exhibit charges ranging from positive to negative, including a zwitterionic characteristic. Functionalisation of nanoparticles is achieved by utilising ligands with desired characteristics. The main interactions that can be induced through ligands are: (i) enabling adsorption of specific proteins through surfactants like polysorbate 80 (P-80); (ii) allowing direct interaction with the blood-brain barrier (BBB) through transferrin proteins, antibodies or similar; (iii) increase the hydrophobic character of the nanoparticles by attaching amphiphilic peptides; or (iv) alter the duration of blood circulation of the nanoparticles, e.g. by binding poly(ethylene) glycol (PEG) to the particle surface (Saraiva et al. 2016).

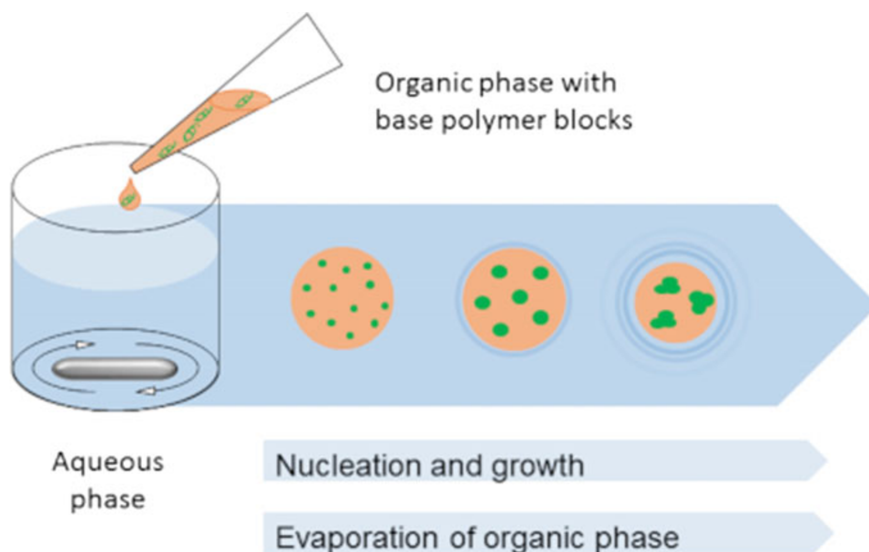

Fig. 2 Schematic illustration of nanoparticle preparation by dispersion method. To prepare nanoparticles, the base polymers are dissolved in an organic phase and then added to an aqueous phase with continuous agitation. Agitation methods can include stirring, ultrasound, mechanical mixing or others. The polymer blocks in the finely dispersed organic phase form nucleation cores. These cores grow by accretion of further polymer blocks to the cores, encouraged thorough continuous evaporation of the remaining organic phase

(Fig. 2). In order to influence the produced particle, different parameters, including the dispersion method itself, the complexity and characteristics of the used solvents, the polymer blocks as well as the ratio of potentially more than one base polymer can be adjusted to achieve a certain aim. For both methods, the drugs to be delivered can be trapped within the developing particle structure or linked covalently or chemically to the particle surface (Vauthier and Bouchemal 2009). The nanoparticle surface also provides an area for further development and specific modifications.

Historically, the first pharmaceutically developed nanoparticles were "bare" on the surface, but already Akasaka et al. (1988) published an albumin-based nanoparticle modified with cell-specific antibodies for targeted delivery. In 2001, the first albumin-based nanoparticles for tumour therapy advanced to clinical studies (Damascelli et al. 2001) and in 2005, the first albumin-nanoparticles were approved by the "Food and Drug Administration" (FDA) as medicinal product, followed by approval through the European Medicines Agency (EMA) in 2008. This drug is called Abraxane, a formulation of HSA nanoparticles containing the cytostatic agent paclitaxel, and is used in cancer treatment (Miele et al. 2009).

Surface modifications can encompass any number of substances, including peptides, proteins, antibodies, small molecules and even polymers different from the base polymers. They can be attached through covalent bonds or simple adsorption and will have different effects, allowing either passive or active targeting approaches,

but also increasing the circulation time of the nanoparticles in the blood stream (Gref et al. 1994). This is necessary to allow sufficient absorption of the nanoparticles in the targeted tissues (Zara et al. 2002) and is achieved by masking the nanoparticles from being recognised as foreign by the immune system (Storm et al. 1995). Potential ways to mask them include hydrophilic surfaces modifications (van Vlerken et al. 2007; Kaul and Amiji 2002) as well as addition of surfactants (Troster and Kreuter 1992) or serum components (Wagner et al. 2012), forming so called "stealth"-particles with increased retention time in circulation (Moghimi and Hunter 2001). Combining this effect with further targeting, either passive or active, provides a highly specific delivery system for drugs.

2.1 Passive Targeting

The passive targeting approach is most common in tumour targeting. Tumour growth is usually accompanied by increased angiogenesis and decreased lymphatic drainage (Maeda et al. 2000). The formed blood vessels tend to be imperfect in as far as the endothelial cells lining the vessels do not form a continuous, unbroken structure. The normal tight endothelial configuration prevents nanoparticles circulating in the blood stream from simply being taken up into the surrounding tissue. When the endothelial barrier is thus riddled with imperfections, the particles can simply penetrate through these into the surrounding tissue. The vessels found in tumours show holes between endothelial cells with a diameter of 200 to 600 nm, thus allowing nanoparticles to be taken up into the tumour tissue and accumulate there (Noguchi et al. 1998). This effect is known as the "Enhanced Permeability and Retention" (EPR) effect and forms the basis for passive targeting. This effect is especially useful when combined with the above-mentioned "stealth"-particles.

2.2 Active Targeting

Active targeting makes use of cellular structures of the target cells and uses established cellular uptake mechanisms for the nanoparticle transport into the cells. Ligands like polysaccharides, proteins or antibodies are bound to the nanoparticle surface and will interact with their specific receptors present on the cellular surface (Nobs et al. 2004). This enables the active uptake of the nanoparticles in the cells of interest. Based on the specific ligand selected for the particle modification, specific cells and tissues can be targeted as destination of the nanoparticles.

3 Nanoparticles for Specific Tumour Therapy

Therapeutic intervention for cancer treatment and tumour targeting is a major area of interest in nanoparticle development. Due to the nature of tumour growths as highly proliferating and adaptable tissues, successful drugs often have a general cytotoxic affect and a wide variety of negative side effects. Encapsulating these drugs in nanoparticles has the potential of masking undesirable side effects while still delivering them to the areas where their effects are of use, namely the tumours themselves. This masking has been shown for the chemotherapeutic doxorubicin, whose cytotoxic side effects on heart tissue and testes were significantly lowered when encapsulated in HSA nanoparticles (Pereverzeva et al. 2007). Examples for tumour specific targeting are the targeting of breast cancer or melanoma cells based on receptor expression and making photosensitisers a viable treatment option despite their challenging side effects.

3.1 HER2-Overexpressing Breast Cancer

The human epidermal growth factor receptor-2 (HER2) is one of three important receptors that can be found in breast cancer and is an important prognostic factor. A humanised monoclonal antibody targeting HER2 called trastuzumab was approved by the FDA in 2008 and improved prognosis of patients with this type of cancer significantly. Modifying nanoparticles with this agent allows for making use of the effect trastuzumab can achieve on its own while simultaneously delivering a second drug to combat the tumour. Incorporating doxorubicin as chemotherapeutic agent into HSA nanoparticles and modifying them on the surface with covalently bound trastuzumab has allowed for specific targeting of HER2-overexpressing breast cancer cells, improved internalisation (Fig. 3) and prolonged the effect of doxorubicin in these cells (Anhorn et al. 2008).

The nanoparticle base of HSA is rapidly degraded after cellular uptake allowing the encapsulated doxorubicin to be released and therefore significantly increasing its intracellular concentration and effectiveness. The uptake into the target cells is insured through the monoclonal antibody trastuzumab. When bound on the nanoparticle surface, the antibodies are still capable of binding to their target receptor HER2 on the tumour cells and are quickly internalised. Due to the covalent bond with the nanoparticles, these are internalised in the same process and thus become accessible for the intracellular degradation and doxorubicin release (Fig. 4).

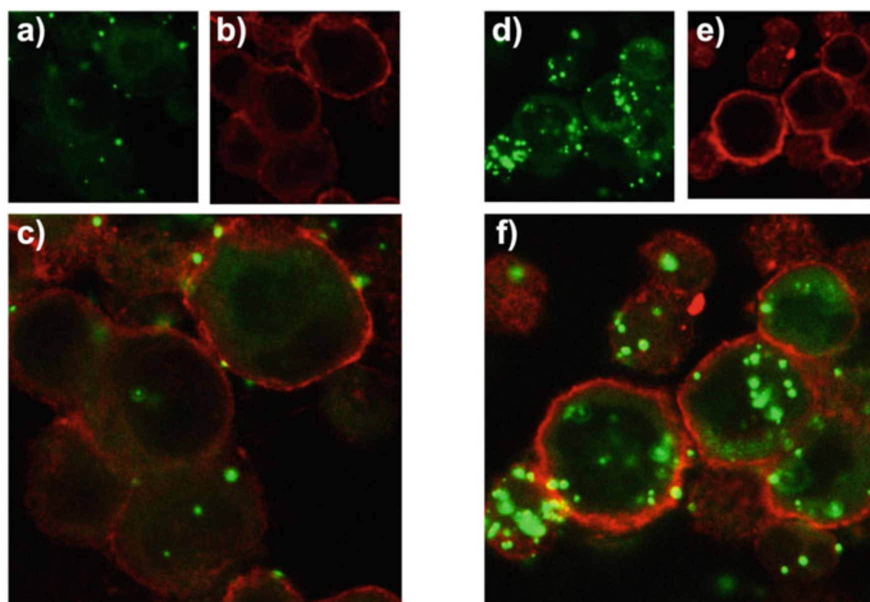

Fig. 3 Cellular uptake and intracellular distribution of HSA nanoparticles analysed by confocal laser scanning microscopy. HER2-overexpressing breast cancer cells SK-Br-3 were cultured on glass slides and treated with either **(a–c)** doxorubicin-loaded control nanoparticles without antibody modification or **(d–f)** doxorubicin-loaded nanoparticles modified with the specific HER2-recognizing antibody trastuzumab for 4 h at 37 °C. Nanoparticles show autofluorescence (green channel, **(a)** and **(d)**) and cell membranes were stained with Concanavalin A AlexaFluor 594 (red, **(b)** and **(e)**). Images **(c)** and **(f)** show an overlay of the dedicated channels. All images were taken of inner sections of the cells (Anhorn et al. 2008)

3.2 Melanomas

The same principles as described above can be used to target any number of different cancers based on the expression of specific receptors. A second example is the targeting of melanoma cells based on their overexpression of integrin $\alpha_V\beta_3$ (Wagner et al. 2010b). This integrin is involved in angiogenesis and is overexpressed in a number of cancers including melanomas. A monoclonal antibody called DI17E6 has been described, that can inhibit the growth of melanomas both in vitro and in vivo. When this antibody is covalently bound to doxorubicin-loaded HSA nanoparticles, its own effect can be enhanced through the cytotoxic effect of the doxorubicin when delivered directly to the melanoma cells (Fig. 5). This leads to an increased cytotoxicity in the target cells ($\alpha_V\beta_3$-high expressing melanoma cells) when compared to either free doxorubicin or the DI17E6 antibody alone, while not showing cytotoxicity in cells low in expressing integrin $\alpha_V\beta_3$, as shown in Table 1:

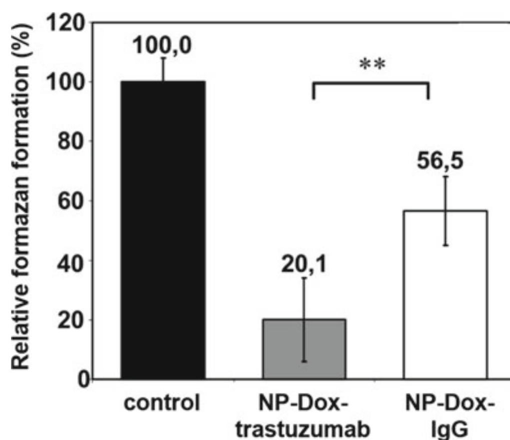

Fig. 4 Cell viability assay comparing doxorubicin-loaded trastuzumab-modified nanoparticles (NP-Dox-trastuzumab) with doxorubicin-loaded IgG-modified control nanoparticles (NP-Dox-IgG). HER2-overexpressing breast cancer cells SK-Br-3 were incubated with the same concentration of either specific NP-Dox-trastuzumab or control NP-Dox-IgG in PBS for 4 h at 37 °C. After washing the cells were incubated for a further 7 d at 37 °C. WST-1 reagent was used to determine the cell viability by measuring the formazan formation. The 100% standard was set with untreated cells (internal control of each experiment n = 9, one representative experiment out of three independent experiments is shown). **: The two samples are significantly different ($P < 0.01$, two-tailed Mann-Whitney U Test equivalent to Wilcoxon rank sum test) (Anhorn et al. 2008)

3.3 Nanoparticulate Photosensitisers for Tumour Therapy

Photosensitisers are compounds capable of producing highly reactive oxygen species and inducing cellular death when exposed to light of a certain wavelength. The second generation photosensitiser mTHPC (trade name Foscan) was approved for the treatment of advanced head and neck cancer by the EMA in 2001 and has shown a significant phototoxicity in many cancer cells in vitro. Yet, due to its poor water solubility and dark toxicity the applicability to a wider variety of cancer in vivo has proven difficult. Therefore nanoparticles were developed encapsulating mTHPC in order to decrease its dark toxicity and improve water solubility. PLGA nanoparticles loaded with mTHPC were shown to accumulate mTHPC in colon carcinoma cells in an amount comparable to the free substance (Löw et al. 2011). While the phototoxic effect after light exposure was similar in cells treated with either the encapsulated or free mTHPC, the dark toxicity was significantly reduced in cells exposed to nanoparticular mTHPC (Fig. 6). Therefore encapsulating mTHPC ameliorates the negative side effects of the drug while maintaining its intended effect. Combining the encapsulation of mTHPC with targeting approaches as discussed above could open up a number of avenues where mTHPC can be used in cancer treatments without the significant side effects that prevent the wider use of the free substance.

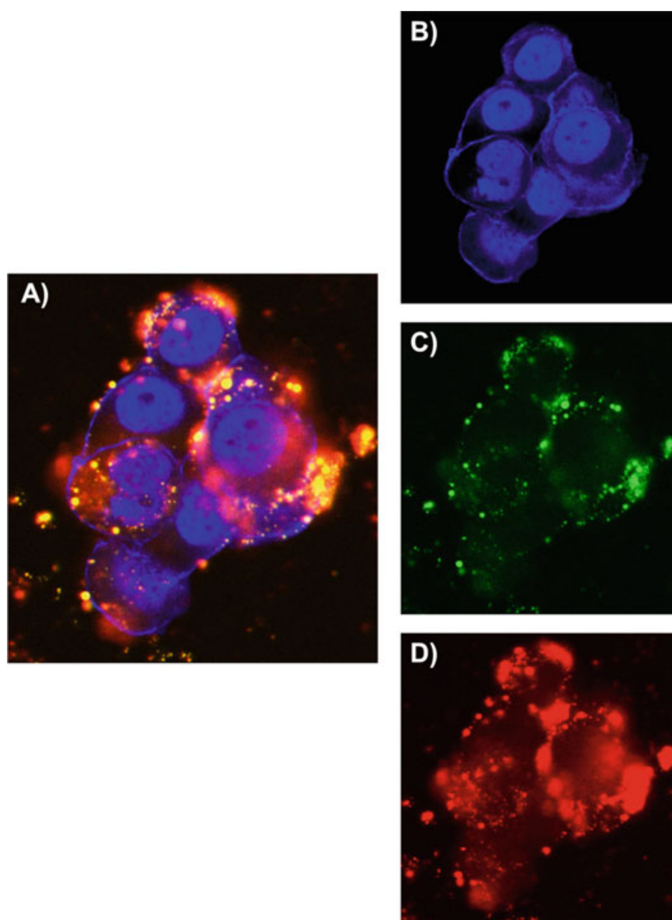

Fig. 5 Cellular uptake and intracellular distribution of doxorubicin-loaded DI17E6-modified HSA nanoparticles studied by confocal laser scanning microscopy. $\alpha_V\beta_3$-high expressing melanoma cells M21 were cultured on glass slides and treated with 10 ng/μL doxorubicin-loaded DI17E6-modified HSA nanoparticles for 4 h at 37 °C. Nanoparticles were visualised using their green autofluorescence, doxorubicin showed red autofluorescence and cell membranes were stained with Concanacalin A AlexaFluor 350 (blue). Images were taken within the inner cell sections. **A** overlay of all channels, **B** cell membranes stained blue, **C** green autofluorescence of HSA nanoparticles, **D** red autofluorescence of doxorubicin (Wagner et al. 2010b)

4 Nanoparticles for Crossing Biological Barriers

Nanoparticular development for improved drug delivery does not only have to take the ultimate target of the particle delivery into account, but also needs to consider the route the particles have to take to reach that target. The initial application of nanoparticles is theoretically possible through all routes also used for non-encapsulated drugs.

Table 1 IC-50 values of different nanoparticulate formulations in $\alpha_V\beta_3$-high expressing melanoma cells (M21) or $\alpha_V\beta_3$-low expressing melanoma cells (M21L) (Wagner et al. 2010b)

	M21 [ng/ml]	M21L [ng/ml]
Nanoparticle preparation		
NP-Dox unmodified	30.8 ± 3.5	75.4 ± 8.3
NP-Dox-Peg	>100	>100
NP-Dox-DI17E6	8.0 ± 0.2	>100
NP-Dox-IgG	>100	>100
Controls		
free doxorubicin	57.5 ± 3.7	70.7 ± 0.8
free DI17E6	>100	>100

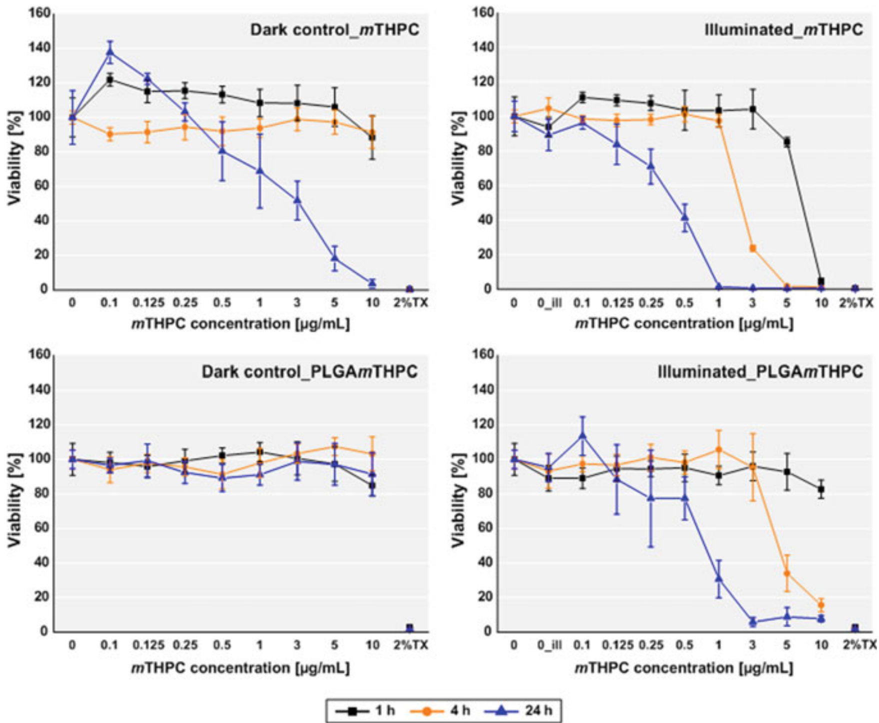

Fig. 6 Determination of cell viability. Colon carcinoma cells HT29 were exposed to varying concentrations of free mTHPC or mTHPC-loaded PLGA nanoparticles (corresponding to a photosensitizer concentration of 0.1–10 µg ml − 1) for 1, 4, and 24 h followed by illumination (652 nm; 5 J cm^{-2}). Control experiments were not illuminated (Dark control). The cell viability was measured by WST-1 assay. Each result represents the mean viability ± standard deviation (SD) of three independent experiments and each of these was performed in biological triplicates. Cell viability was calculated as percentage of viable cells compared to untreated control cells. Untreated cells were used as negative control (0; 0_ill:illuminated control cells) and 2% Triton X-100 (2% TX) treated cells as positive control (Löw et al. 2011)

The main routes used are topical, oral or intravenous delivery. Especially in the case of topical and oral delivery, additional barriers have to be overcome for the nanoparticles to reach the blood stream. Next to topical administration, oral delivery has high patient compliance and is therefore the preferred mode of administration when it comes to a wide variety of drugs. The absorption occurs in the gastrointestinal tract, mainly the duodenum and small intestine, as long as the nanoparticles are capable of interacting with this gastrointestinal barrier (Ensign et al. 2012).

4.1 The Gastrointestinal Barrier

The gastrointestinal tract provides a significant barrier for the absorption of drugs and nanoparticles. Different areas have different pH values, which affect the chemical properties and thus absorption rates of substances based on their structure and properties. Additionally, the intestinal tract is covered by a mucus layer of varying thickness depending on the area of intestine. To either overcome or utilise this mucus barrier, administered nanoparticles need to be specifically modified to either attach to or cross the mucus layer (Ensign et al. 2012). Successful mucus penetration or permeation has been shown with PLGA nanoparticles loaded with mTHPC as a model drug and duodenal cancer as target (unpublished data). The nanoparticles were modified with specific surfactants to change their surface properties in such a way, that they were preferentially bound to the intestinal mucus. Their size in combination with physical characteristics then allowed them to either penetrate the mucus layer and reach the underlying duodenal cells, or caused them to be stuck in the mucus layer and degraded there, releasing the contained drugs at this specific side. The produced nanoparticles showed increased accumulation in cellular models of duodenal cells with a mucus layer in contrast to intestinal cells without this layer.

Excursion: The Mucus Chip—An Initial Advanced Testing Module
Considering both the complexity of the gastrointestinal system in general and the challenging nature of the intestinal mucus layer specifically, advanced testing system are necessary to investigate the properties and behaviour of orally administered nanoparticles (Elberskirch et al. 2019). As the first hurdle to either encountering intestinal cells or absorption into the general circulation is the mucus layer itself, a specific test system for mucus penetration and retention was devised. The mucus chip contains a defined layer of intestinal mucus suspended between permeable membranes (Fig. 7). To simulate the intestinal situation, substances are applied from the apical side in a static system, while a peristaltic flow of buffer solution is maintained on the basolateral side to simulate natural peristalsis. Based on the amount of nanoparticles found in the basolateral compartment at different time points, the mucus permeation can be measured and further optimisation steps considered.

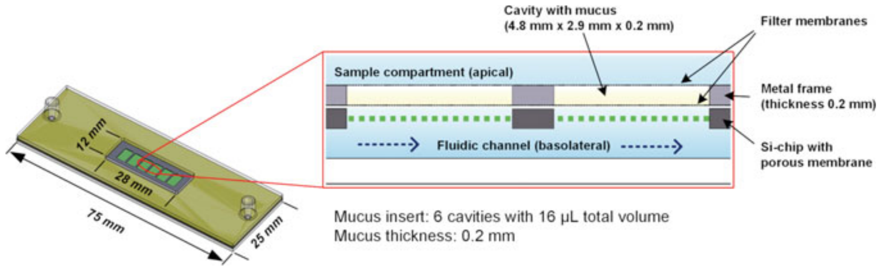

Fig. 7 Microfluidic mucus-chip module. **A** Assembled cartridge with mucus insert, **B** cross-section view of mucus insert, micro-membrane chip and compartments (Elberskirch et al. 2019)

4.2 *The Blood-Brain Barrier*

Another region of the human body, that poses a significant challenge for drug delivery, is the brain. Any drugs, substances or nanoparticles, that want to reach brain tissue, have to pass the blood-brain barrier (Pardridge 2005). This barrier is comprised of the endothelial cells forming the brain capillaries, the surrounding basement membrane with embedded pericytes and the end feet of associated astrocytes (Keaney and Campbell 2015; Fig. 8). What makes the capillaries within the brain unique in contrast to the

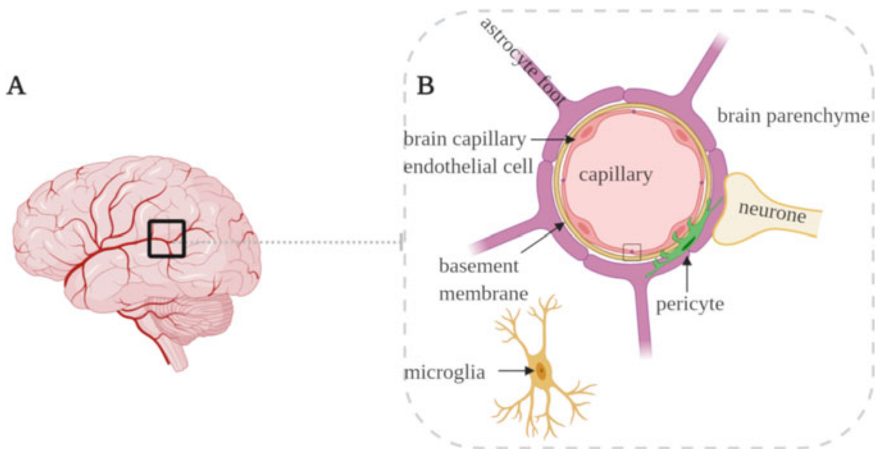

Fig. 8 The human blood-brain barrier. **A** An overview of the human brain with vasculature and **B** a graphical representation of the organisation of cells surrounding brain capillaries, known as neurovascular unit. The capillary wall is comprised of brain capillary endothelial cells (red) connected by tight junctions (little square). The endothelial cells are surrounded by a basement membrane (yellow), in which pericytes (green) are embedded. On the brain side of the basement membrane, the capillaries are encompassed by the endfeet of astrocytes (purple), which can be connected to neuronal axons (beige). The only cell type not directly associated with the neurovascular unit are microglia (orange) present in the brain parenchyma. Created with BioRender.com

rest of the body is the fact that the endothelial cells are connected by tight junctions all around, leaving no gaps at all and restricting even small molecular diffusion in the intracellular space (Daneman and Prat 2015). The main options to overcome this barrier are active or passive transport processes. Only a small number of either water- or lipid-soluble molecules can cross the blood-brain barrier independently. The transport processes used for uptake are adsorptive transcytosis, receptor-mediated transcytosis or the use of transport proteins (Saunders et al. 2013; Tuma and Hubbard 2003). Nanoparticles, due to their size, are restricted to making use of one of these processes. The exact route nanoparticles take in order to reach neuronal cells has been extensively discussed and investigated (Wohlfart et al. 2012; Wagner et al. 2012). It has been shown that nanoparticles modified to present apolipoprotein E (ApoE) on their surface are internalised into brain capillary endothelial cells through receptor-mediated endocytosis by the LRP1 receptor, as shown through mechanism studies (Wagner et al. 2012), followed by transcytosis to neighbouring cells, specifically astrocytes (Begley 2012), and can be found in different regions of the brain as soon as 30 min after intravenous injection (Fig. 9) in a mouse model (Zensi et al. 2009). ApoE or other ligands like ApoA1 (Zensi et al. 2010), transferrin (Ulbrich et al. 2009) or insulin (Ulbrich et al. 2011) have dedicated receptors expressed in the brain capillaries. These receptors can be targeted for nanoparticle transport using specific antibodies for these receptors similar to tumour targeting (Ulbrich et al. 2009) or by presenting the receptor-specific proteins on the nanoparticle surface. To achieve this, the proteins can be covalently bound or non-covalently recruited to the nanoparticles. Specific modifications are necessary to achieve this and examples are shown in the following paragraphs.

4.2.1 Covalently Bound ApoE

ApoE can be bound to the surface of different base nanoparticles and used for a variety of treatments.

In one study, HSA nanoparticles with covalently bound ApoE have been developed and used to transport oximes across an in vitro blood-brain barrier model (Wagner et al. 2010a). Oximes are used in the treatment of organophosphate poisoning by reactivating the blocked acetylcholine esterase in cells, but cannot cross the blood-brain barrier in therapeutically relevant doses. Thus, they are unable to ameliorate the neurological effects caused by an imbalance in acetylcholine as neurotransmitter. Therefore, they were adsorbed to HSA nanoparticles. These particles had ApoE bound to their surface and were then applied to an in vitro blood-brain barrier model. It was shown that only particles with the ApoE modification were taken up into the capillary endothelial cells in a significant amount. These nanoparticles were also capable of transporting oximes across the in vitro barrier model while maintaining their functionality in combating organophosphate poisoning (Dadparvar et al. 2011).

In a second study, the base particle consisted of PLA (Stab et al. 2016). Here, the ApoE-modified nanoparticles showed as well higher accumulation in brain capillary endothelial cells than unmodified nanoparticles (Fig. 10).

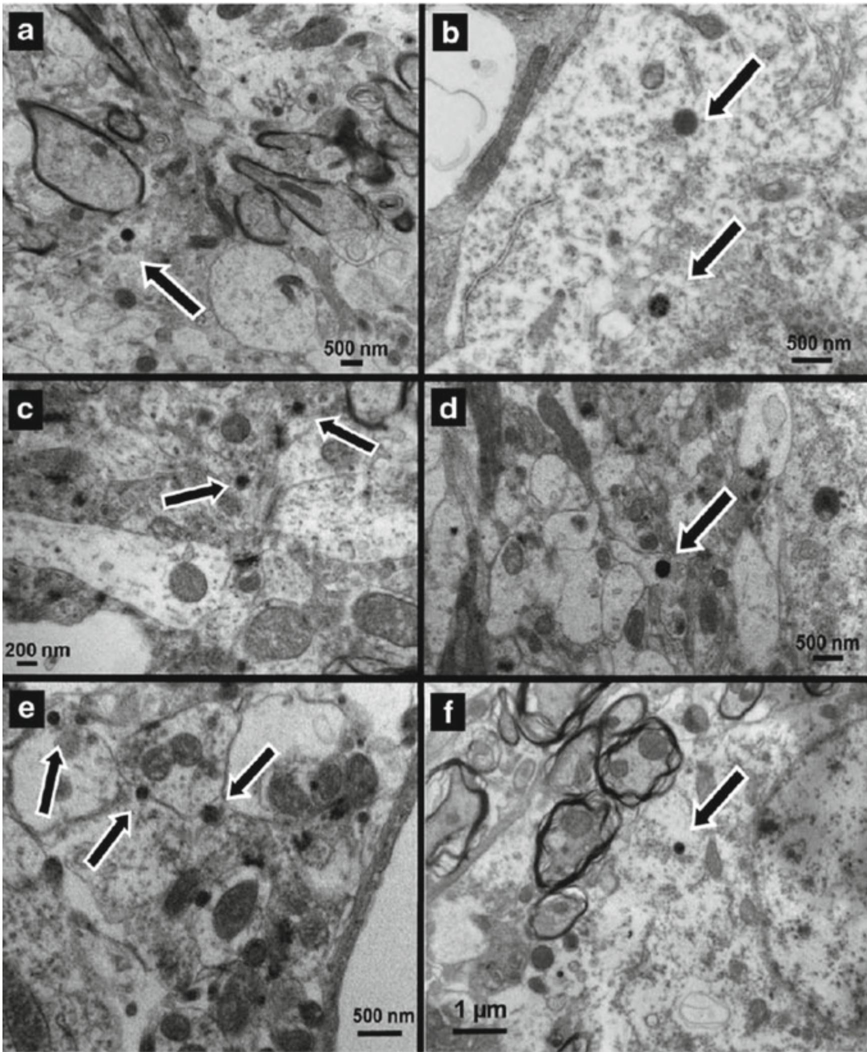

Fig. 9 Apo-E modified HSA nanoparticles in different brain regions in a SV 129 mouse model. SV 129 mice were injected in the tail vain with 200 μg ApoE-modified HSA nanoparticles per g body weight. 30 min after application transcardiac perfusion with a fixative was initiated, brains removed and sliced. Samples corresponding separate sections of the brain were embedded in resin and stained with uranyl acetate for electro microscopy. Nanoparticles are indicated by arrows and were found in **a** the olfactory bulb, **b** the cortex, **c** the striatum, **d** the hippocampus, **e** the cerebellum and **f** the brain stem (Zensi et al. 2009)

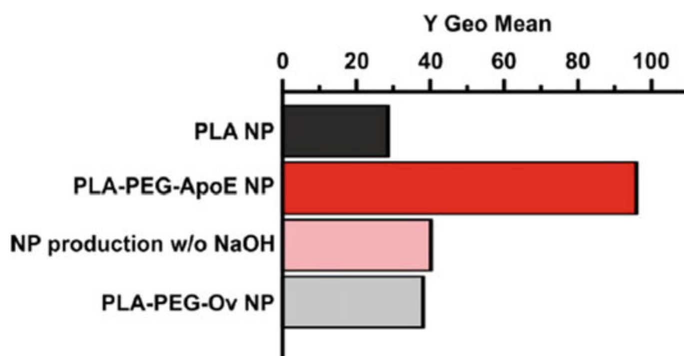

Fig. 10 Influence of ApoE3-modification on binding and uptake characteristics of nanoparticles studied via flow cytometry experiments. Primary porcine brain capillary endothelial cells pBCEC were incubated for 4 h at 37 °C with unmodified control nanoparticles (PLA NP); ApoE3-modified nanoparticles (PLA-PEG-ApoE NP); ApoE3-modified nanoparticles prepared without NaOH in the buffer (NP production w/o NaOH) or nanoparticles modified with the control protein ovalbumin (PLA-PEG-Ov NP). Binding intensity was assessed by measuring the Y Geo Mean (Stab et al. 2016)

4.2.2 Non-covalent ApoE Recruitment

The protein ApoE cannot only be bound to nanoparticles before they are administered, it is also present in human serum and circulates freely in the blood stream. Thus, if a reliable way to attract it to the surface of circulating nanoparticles is identified, the natural reservoir of this apolipoprotein can be used for targeting the nanoparticles. Meister et al. (2013) have shown that PLA nanoparticles produced in the presence of polyvinyl alcohol (PVA) are capable of attracting and maintaining a so-called protein corona of plasma proteins. This corona contained both ApoE and ApoA1 among other proteins. Flurbiprofen-loaded PLA-PVA nanoparticles were successfully taken up into brain capillary endothelial cells, capable of crossing an in vitro blood-brain barrier model and could reduce the γ-secretase activity though in vitro release of the contained flurbiprofen (Meister et al. 2013). They were also capable of decreasing the amount of $A\beta_{42}$ produced by an in vitro cellular model of Alzheimer's disease (Stab et al. 2016, Fig. 11).

Excursion: Blood-Brain Barrier Model Systems

The decision, whether a covalent or non-covalent binding of targeting modifications is necessary or advisable needs to be made on a case by case basis. Every particle system is unique in its properties and behaviour dependent on particle material, contained substance and targeted application. To analyse and evaluate this, reliable, advanced model systems for the targeted areas are vital. This is especially true in the case of the blood-brain barrier. Reliable, reproducible model systems closely mimicking the in vivo situation and reactions are essential for evaluating nanoparticles developed for BBB crossing (Helms et al. 2016). Initial model systems were based on immortalised cell lines and maintained the receptor and transporter status of the in vivo BBB, but

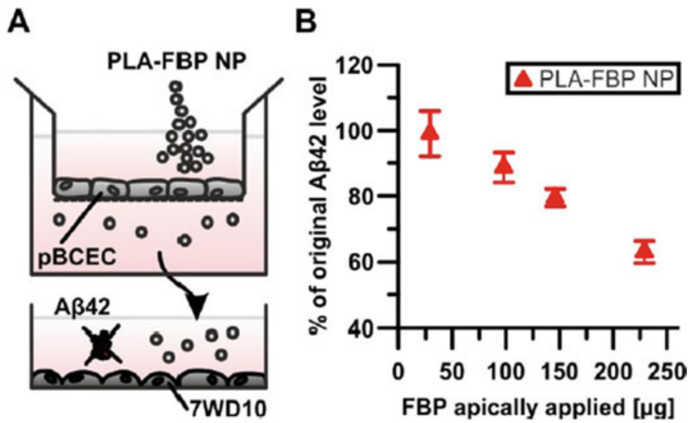

Fig. 11 Aβ42-lowering capacity of nanoparticles after in vitro BBB Crossing. **A** Schematic drawing of experimental design: Primary porcine brain capillary endothelial cells pBCEC were cultivated on transwell inserts simulating the BBB. When Transendothelial Electrical Resistance (TER) was adequate, drug-loaded nanoparticles (PLA-FBP NP) were added for 4 h. Then, the apical compartment and pBCEC were discarded and basolateral medium was transferred to culture plates seeded with Aβ42 producing Alzheimer's disease model cells 7WD10 for 72 h. **B** Analysis of 7WD10 supernatants by a human Aβ42 recognizing ELISA assay. Data from at least 3 independent experiments (Stab et al. 2016)

the characteristic barrier tightness could not be replicated. This barrier tightness is characterised by the transendothelial electrical resistance (TER) value, which can be determined and monitored in model systems. Immortalised cell lines achieved at most values of 40 Ωcm^2. As achieving a certain tightness or TER value is one basis for the selectivity, these lines were useful for binding and uptake but not transport studies. A step towards replicating the barrier transport behaviour was taken with the development of in vitro BBB models based on isolated primary cells (Nakhlband and Omidi 2011; Stab et al. 2016). These capillary endothelial cells were isolated from animal sources like bovine, porcine or rodent material. But these models, due to their non-human origin, do not express the identical receptors and transporters as found in humans. A model system presenting all relevant characteristics, both in protein expression and transport behaviour of an intact, sufficiently selective barrier has been published in recent years (Lippmann et al. 2012). The specifically necessary brain capillary endothelial cells are differentiated from human induced pluripotent stem cells (Fig. 12). This human origin ensures a high transferability of the results obtained in this model system based on transporter status and the barrier tightness obtained is comparable or superior to that achieved in primary cell models (Lippmann et al. 2014). The source material of human induced pluripotent stem cells also presents the opportunity to develop specific models for neurological diseases and investigate whether the uptake and transport processes are altered in a diseased state and if so, how this impacts therapeutic options.

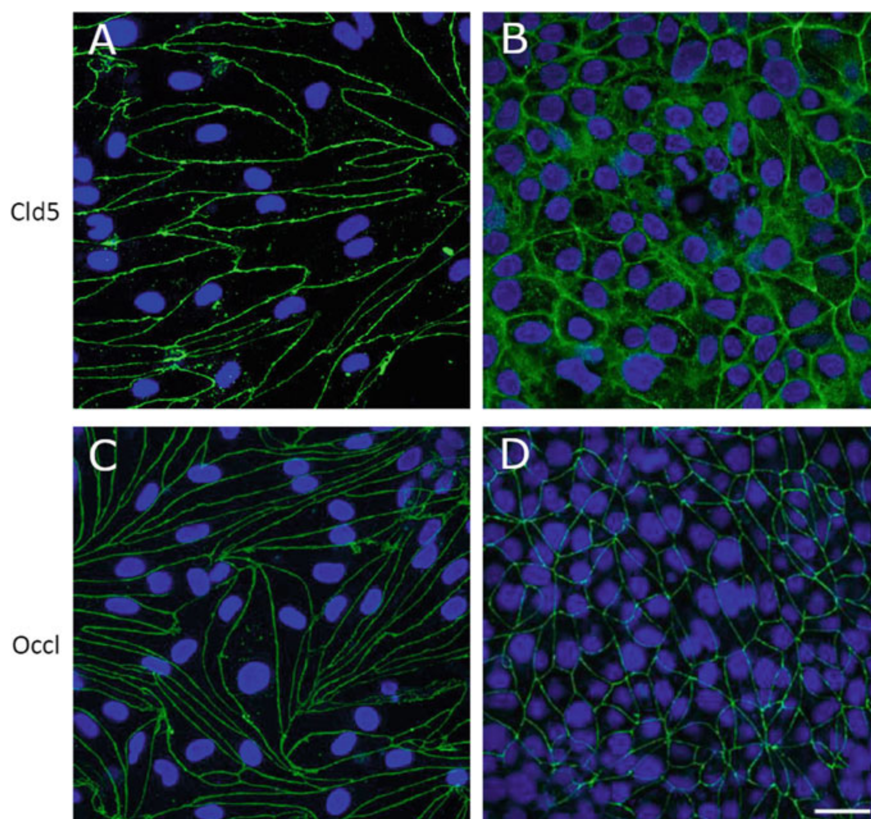

Fig. 12 Comparison of protein expression of primary porcine- and human induced pluripotent stem cell-derived blood-brain barrier models by confocal laser scanning microscopy. Primary porcine brain capillary endothelial cells (**A** and **C**) and stem cell-differentiated brain capillary endothelial cells (**B** and **D**) were cultivated on polycarbonate membrane inserts with 3.0 μm pores for 3 days at 37 °C before fixation with an aceton:methanol (7:3) mixture. Separate sections of the inserts were stained (A, B) for Claudin-5 (Cld5) with a rabbit anti-Claudin-5 antibody and (**C**, **D**) for Occludin (Occl) with a rabbit anti-Occludin antibody before counterstaining with a goat anti-rabbit AlexaFluor 488 antibody (green). Nuclei were DAPI stained (blue). Membranes were immobilised between two cover slides for microscopic analysis. Images **A** and **B** show Claudin-5 (green) and DAPI (blue) staining, images **C** and **D** show Occludin (green) and DAPI (blue) staining. Size bar 25 μm

5 Final Remarks

Overall, it is clear that nanoparticles are a highly adaptable option for drug formulations capable of masking undesirable side effects of drugs while allowing them to maintain their therapeutic effect upon release in the target cells or tissue. Especially when it comes to at present unsolved issues, like the dark toxicity and hydrophobic characteristics of mTHPC or the selectivity of the blood-brain barrier preventing

most drugs from crossing into the brain, nanoparticles open up new avenues for the application of existing substances or advancing the development of new therapeutic treatments. Based on the possibilities to modify just about every characteristic of a nanoparticle, they can be tailored to achieve specific effects and present targeted properties. But nanoparticles are not limited to applications in drug delivery. Depending on what is incorporated, nanoparticles can be used in theragnostics (Fang and Zhang 2010), diagnostic applications (Mirabello et al. 2015), for gene therapy (Cullis and Hope 2017) or to improve stem cell differentiation (Dayem et al. 2016).

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Conflict of interest The authors declare no conflict of interest.

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