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Local drug delivery systems for enhancement of collateral growth

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Abstract

Drug delivery systems are useful tools for local drug delivery in the enhancement of collateral growth (angiogenesis and arteriogenesis). These systems have major clinical potential for patients suffering from coronary artery disease or peripheral artery disease for whom current therapies do not seem to work sufficiently. Administration of drugs for enhancing collateral growth has been used in the past. However, free drug administration may lead to harmful side effects, such as local edema. Therefore, encapsulation of drugs into carriers leads to improved local delivery with its therapeutic effects on collateral growth.

Different nano- and microparticles have been developed in the past for drug loading and local drug delivery. These carriers can move through the vasculature without being obstructed. Therefore, they can reach any desired area for stimulation of collateral growth. One type of microparticle is the microbubble (MB), which has been frequently tested in animal models. Different constructs have been developed already for carrying different drug types, such as proteins and gene constructs for cell transfection. Lipid-coated and polymer-coated MBs have been developed in the past.

In this thesis, different drug delivery systems will be evaluated for the enhancement of collateral growth. Current therapies tested in experimental studies and clinical trials will be evaluated. The main focus is on new therapeutic approaches for local and sustained drug delivery. Furthermore, suggestions are made for MB constructs to optimize drug delivery for the enhancement of collateral growth.

Background

In 2008, according to the World Health Organization, cardiovascular disease (CVD) was still the number one cause of death in the European Western society. Coronary artery disease (CAD) and peripheral arterial disease (PAD) are two subsets of CVD affecting the arteries throughout the body. Well-known causes of CVD are an unhealthy diet, low physical activity and its associated obesity, and tobacco use[1]. The prevalence of CVD, and its associated morbidity, still remains high. Standard therapies for patients suffering from CVD are quite invasive. Current therapies used in the clinic are bypass surgery or, less invasive, percutaneous transluminal angioplasty[2]. However, in 20 % of the cases, these therapies are not beneficial or even not a clinical option[3]. Over the past decade, the percutaneous therapies have evolved enormously, and resulting from this, fewer patients suffer from serious complications. Still, current surgical interventions are associated with high morbidity and mortality rates[4]. In the case of CAD and PAD, the body experiences low perfusion levels in organs that are supplied with oxygen by affected arteries. Neovascularisation is the body's most important attempt for restoration of tissue perfusion to normal levels. This is the formation of new blood vessels in the body, also known as 'angiogenesis'. Unfortunately, this response usually is inadequate to prevent serious tissue damage[5]. Natural collateral artery growth, known as 'arteriogenesis', is another attempt to restore oxygen levels. Stimulation of both processes would be of potential benefit for patients not suitable for current therapies[2]. It is of great importance that these potential new therapies are targeted properly. In the past decade, therapies targeting stimulation of angiogenesis and arteriogenesis have been investigated in both experimental studies and clinical trials. Most studies focussed on administration of growth factors to stimulate collateral growth[6,7]. Although experimental studies showed promising results, application in clinical trials still seems to fail.

In this thesis, different ways of drug delivery for enhancement of collateral growth (angiogenesis and arteriogenesis) will be evaluated. Current therapies tested in experimental studies and clinical trials will be mentioned. The main focus will be on new therapeutic approaches for local and sustained drug delivery. Suggestions will be made for future experimental and clinical therapies to optimize local drug delivery and enhance collateral growth.

Mechanisms of collateral growth

The growth and remodelling of new collaterals is a constant process in the human body.

The earliest process is known as 'vasculogenesis' and is mostly occuring in the early embryonic phases. This process starts with the differentiation of mesodermal cells into angioblasts, which are precursors for endothelial cells. Mesodermal cells home in the bone marrow. The angioblasts differentiate into blood islands, which fuse together to form the primitive capillary plexus. After the formation of this plexus, the process of vascularisation is taken over by capillary sprouting. This process is also known as 'angiogenesis' and occurs both during embryogenesis as well as in adult life[7,8]. Angiogenesis is involved in various normal and pathological processes, such as wound healing, pregnancy, and ovulation. Tight regulation is needed, since it can contribute in processes such as tumour growth and metastases[2,8]. Angiogenesis is triggered by hypoxia and ischemia, resulting from the acute or chronic occlusion of an artery. This occlusion leads to defective oxygenation of tissues distant from the occlusion. As a result of defective oxygenation, the ischemic tissue activates and expresses the nuclear factor hypoxia-induced factor 1 (HIF-1). Its expression will lead to transcription of genes for vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), for instance. VEGF is a known mitogen for endothelial cells and is of critical importance in physiological and pathological angiogenesis. VEGF triggers endothelial cell proliferation and therefore promotes budding, sprouting and formation of new capillary networks[2,7]. In addition to its mitogenic effect on endothelial cells, VEGF also has other pro-angiogenic effects. It is known to act as a chemo-attractant on monocytes and macrophages and therefore holds pro-inflammatory properties, which are of importance in angiogenesis[10]. Another well-known factor involved in angiogenesis is the FGF family, which consists of 9 subtypes. FGFs are mitogens for endothelial cells and SMCs. FGF-1 and FGF-2 are commonly known in angiogenesis. They are chemotactic for endothelial cells and therefore stimulate their migration towards areas of developing arteries[10]. Other known stimulators of angiogenesis, although less frequently cited in literature, are interleukin-8, MMPs, and NOS[6].

The process of 'arteriogenesis' is rapidly triggered, when a major artery has a chronic or acute obstruction. In this process, pre-existing collaterals have the ability to increase in size and lumen to provide enhanced perfusion at places where it is requested. Arteriogenesis can be observed in two phases. In the proliferation phase, tissue destruction takes place to create space for the growth and expansion of the collateral artery. In the remodelling phase, the expanded artery stabilizes with newly formed cell layers[8].

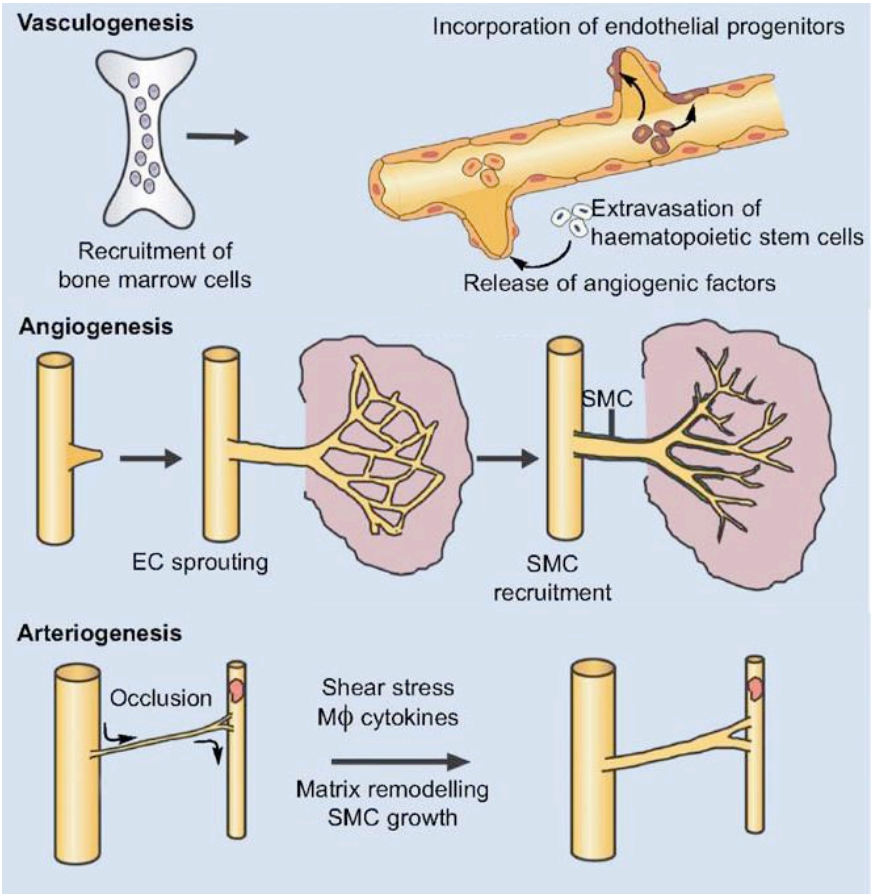

Figure 1. Mechanisms of collateral growth. Vasculogenesis involves homing of angioblasts from bone marrow, formation of capillary plexus and formation of a network via capillary sprouting. Angiogenesis involves growth and enlargement of capillaries by SMC recruitment. Arteriogenesis is initiated by shear stress due to an obstruction in the major artery and involves outward remodelling of pre-existing anastomoses. Inflammation is an important factor in arteriogenesis (adapted from Fischer et al, 2006, Handbook of Experimental Pharmacology[9]).

Arteriogenesis is a non-hypoxic event and can occur in normoxic tissues. The process is initiated by shear stress in pre-existing capillaries, due to an occlusion in the major artery. In arteriogenesis, pre-existing collateral arteries develop into functional collateral arteries, when triggered[2]. It has been shown that collaterals continue to remodel even when increased shear stress is no longer present. This indicates that shear stress is only involved in the initiation of the process[11]. After shear stress diminishes, most collateral arteries degenerate and some larger arteries continue to increase in size[12]. Inflammation is a crucial process for arteriogenesis as well. When the endothelium in the collateral artery is activated by increased shear stress, adhesion molecules on endothelial cells are upregulated. Chemokines, such as monocyte chemo-attractant protein-1 (MCP-1), are also expressed by the endothelium. As a

response to MCP-1, monocytes will infiltrate into the perivascular space of the vessel wall and differentiate into macrophages. The initiated inflammatory process will further develop and attract more inflammatory cells due to cytokine and chemokine production, such as more MCP-1, transforming growth factor-β (TGF-β) and granulocyte-monocyte colony-stimulating factor (GM-CSF). Increasing inflammation will lead to enlargement of collaterals[7]. MMPs produced by inflammatory cells are involved in the remodelling of the pre-existing collateral artery to create space for the incoming inflammatory cells for expansion of the collateral artery. VEGF and FGFs are also involved in arteriogenesis[2,8]. A schematic overview of these processes can be observed in figure 1.

Therapeutic approaches for stimulation of collateral growth in experimental studies

Therapeutic stimulation of angiogenesis and arteriogenesis has been extensively studied in experimental studies. VEGF and FGF have been used mostly and are administered in different ways and via different routes. Other growth factors also seem to have a stimulating effect on both processes. Animal models for testing in CAD, used a myocardial infarction model induced by the ligation of one of the coronary arteries. For PAD, the hind limb ischemia model is used.

Administration of human VEGF showed promising results for stimulation of angiogenesis and collateral blood flow in animal models for myocardial infarction[13,14]. In animal models for hind limb ischemia improved tissue perfusion and visible angiogenesis were observed in ischemic tissues[14,15]. FGF-2, also known as basic FGF (bFGF), is also an angiogenic factor. In animal models for myocardial infarction, infusions of bFGF resulted in a reduction of infarct size, an increase of collateral formation, improved myocardial function, and increased perfusion of the myocardium after administration[13,14,16].

Next to VEGF and bFGF, other factors, such as cytokines and chemokines, stimulating angiogenesis are known. Examples are IL-8, MCP-1, MMPs, TNF-α and TGF[6].

Arteriogenesis can also be stimulated by different factors, such as growth factors, cytokines and chemokines in experimental studies. Colony-stimulating factors play an important role in arteriogenesis, since they are involved in proliferation and differentiation of different leukocyte subsets. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is used for arteriogenesis stimulation[2,17]. In pigs, administration of GM-CSF in the hind limb after femoral artery occlusion resulted in the stimulation of arteriogenesis[18]. Granulocyte colony-stimulating factor (G-CSF) stimulates arteriogenesis to improve myocardial functioning after

myocardial infarction in mice[19]. Transforming growth factor- β (TGF-β) is highly expressed at sites of collateral development. In a rabbit model for hind limb arteriogenesis, it has been shown to stimulate this process. It has even been suggested that TGF-β holds an athero-protective and plaque stabilizing effect[7].

FGFs are also well investigated growth factors for arteriogenesis. In animal models for myocardial infarction and hind limb arteriogenesis, bFGF treatment stimulates collateral flow and arteriogenic remodelling[20,21,22]. VEGF holds these properties as well. In a rat model for hind limb arteriogenesis, VEGF significantly stimulated microvascular blood flow and vessel density[5]. Simultaneous delivery of bFGF and VEGF even resulted in additive or synergistic effects in the stimulation of arteriogenesis[23]. Other stimuli for arteriogenesis are TNF-α[24] and IL-20[25].

To conclude, both VEGF and bFGF are extensively studied and seem to stimulate angiogenesis and arteriogenesis in different animal models for CAD and PAD. In arteriogenesis, other stimuli, like cytokines and chemokines seem to be good candidates for its stimulation, because of their ability to attract inflammatory cells.

Therapeutic approaches in clinical studies

Administration of different stimulating factors has been tested already in clinical trials as well. For stimulation of angiogenesis, mainly VEGF and bFGF have been used in patients suffering from CAD or PAD. Contradicting results are emerging from these studies and no clear statements can be made for what is beneficial for stimulation of angiogenesis in patients[26].

In the stimulation of arteriogenesis, clinical studies are also frequently reported. Recently, Grundmann et al.[7] and Schirmer et al.[17] both reported a list of clinical trials that study patients suffering from either CAD or PAD and use different stimulators of arteriogenesis in these trials. Different outcomes can be observed for different growth factors used for treatment, varying from no effect to small clinical improvements. For example, GM-CSF and G-CSF are tested in clinical trials and seem to be beneficial for enhancement of arteriogenesis in patients suffering from CAD or PAD. Clinically, GM-CSF is particularly interesting, because of its ability to lower plasma cholesterol levels and it has shown to reduce plaque surface in a rabbit model[27]. In CAD patients both growth factors have shown to be beneficial when administered locally, via intracoronary or subcutaneous injection. In patients suffering from PAD, GM-CSF treatment did not show any positive results yet[28]. Other factors, such as bFGF, have also been tested clinically. Positive effects of bFGF treatments were only observed in PAD patients and

not in CAD patients[2,17]. Large differences can be observed between the clinical trials designed for analysis of stimulation of angiogenesis and arteriogenesis.

Differences between experimental and clinical trial designs

Translating experimental data into clinical trials still shows difficulties. This can be a result from the differences between the designs of experimental studies and clinical trials. An important difference lies in the animal models used in experimental studies. They do not completely reflect on the ethiopathology of the disease process in humans. Therefore, results from experimental studies do not have a high predictive value for whether the treatment would be beneficial in the clinical situation[29]. Nevertheless, animal models are still useful tools for the initial testing of treatment options for diseases. They will provide unique insights in the pathophysiology and causes of diseases and will often reveal novel targets for treatment[30].

Patients suffering from CAD and PAD are usually dealing with other cardiovascular problems as well. For example, elderly patients, patients suffering from diabetes mellitus, high ox-LDL levels or hyperlipidemia can suffer from serious side effects from the treatments. Stimulating angiogenesis and arteriogenesis could influence formation of atherosclerotic lesions as well, because these disease mechanisms are closely related. When stimulating collateral artery growth, unwanted side effects can always affect the clinical outcome of the patients, which is not reflected in animal models[2].

Drug delivery is also an important factor in the stimulation of angiogenesis and arteriogenesis. It could be an explanation why clinical trials do not show the same results as seen in experimental studies. In experimental studies different drug delivery routes have been tested for stimulation of angiogenesis and arteriogenesis. Known from literature, a local and continuous drug delivery seems to have the most beneficial effects in stimulation of collateral growth, compared to all others, i.e. intravenous, intramuscular, or intrapericardial[7]. In clinical trials, this way of drug delivery may work as well.

In a large dog study to enhance collateral growth after myocardial infarction, four different routes of bFGF administration were compared. These routes were single bolus injection in a central vein, intravenous injection, pericardial injection, and intracoronary injection. The continuous intracoronary, and thereby local, drug delivery was the most effective way to enhance collateral growth locally in these animals[31]. Lazarous et al. focussed on the pharmacodynamics of bFGF after different routes of administration. Intracoronary administration resulted in the highest recovery of bFGF of 3-5%, as compared to left arterial

administration (1.5%) and intravenous or subcutaneous administration (both 0.5%). An explanation might be that bFGF first passes the lungs after intravenous and subcutaneous injection. bFGF uptake by the lungs might limit myocardial bFGF availability after injection.[32]. Continuous drug delivery has been tested before, without using repetitive local drug injections. Locally inserting a stent in an artery eluting a drug is used to imitate local and continuous drug delivery. In a rabbit study, a TGF-β1 eluting stent placed in the femoral artery resulted in stimulation of collateral growth. In another study, GM-CSF stimulated arteriogenesis in pigs by using infusion pumps for intra-arterial delivery, a method also useful for local and sustained delivery of a drug[18,33].

In clinical studies, placing stents or other devices in healthy or vulnerable vessels is not an option. Therefore, drug delivery systems must be developed that are acceptable in the clinic and deliver drugs locally and in a sustained level for stimulation of collateral growth. These systems must be easy to deliver and be as non-invasive as practically possible.

Drug delivery systems

Developing new ways to deliver drugs to enhance collateral growth is important for treatment of acute and chronic tissue ischemia. Angiogenesis and arteriogenesis are both desirable goals for this treatment. Stimulation of collateral growth needs to be a local process and would demand a prolonged administration of stimulating factors in the targeted tissue for optimal results. Development of these new drug delivery systems seems to be a difficult task[34].

A specific drug delivery system needs to be developed that is suitable for sustained treatment to maintain blood or tissue levels of a certain drug for an extended period of time. This system needs to introduce a kind of kinetics in which the drug is released over a longer period of time, systemically or locally. Ideally, the drug needs to be at a constant concentration during its release[35]. Drugs can either be delivered via protein delivery or gene transfection in target cells. For direct delivery of drugs, for instance, proteins, peptides, vaccines, antigens or growth factors can be used[36]. For gene delivery, plasmid DNA, viral vectors (adeno-, adeno-associated, lenti- and retroviruses), or RNA interference can be used[34]. These are being transfected in the target cells for local expression of the specific gene.

<u>Drug delivery particles</u>

Delivery of the drugs (e.g. via proteins or genes) can be achieved via specific carriers in which the drugs can be incorporated. Examples of these carriers are nanoparticles (NPs), ranging in diameter from 10-1000 nm and microparticles (MPs), ranging from 1-250 μm in

diameter. These particles can be delivered via different routes directly into the body. NPs are mostly delivered via intravenous injection, whilst MPs are delivered via intramuscular or subcutaneous injection[36]. Microbubbles (MBs) are small (1-8 μm in diameter) MPs with a gas core and are very frequently cited in literature for drug and gene delivery. Because of their small size, MBs are able to be injected intravenously and can move through the lungs and vasculature, including capillaries, without being obstructed. The gas core can be surrounded by a protein (mostly albumin), lipids, surfactant, or a biocompatible polymer shell. Lipid- and polymer-coated MBs are most frequently used for drug delivery. On the shell of the MBs, specific antibodies can be attached for better recognition of the targeted site. For instance, antibodies can be attached that recognize certain adhesion molecules that are upregulated by damaged endothelium during pathological events. Several studies have shown a significantly enhanced binding of MBs to activated endothelium by targeting different endothelial adhesion molecules using antibodies[37].

Microbubble subtypes

Different MBs have been developed all with different molecular weights and physiochemical properties. Two types of MBs are used in experimental studies thus far, using different types of coatings. These coatings are lipid coating or a polymer coating.

MBs coated with a lipid monolayer are usually used for the delivery of hydrophobic drugs. The thin lipid shell can, however, not prevent leakage of the drug from the bubble during circulation near non-targeted tissues. Using a thick lipid coating of triglycerides, in which the drug can be dissolved, may prevent premature delivery. MBs can also be coated with a polymeric shell. This is a thicker and harder shell than the lipid monolayer. This ensures a much higher drug loading capacity and protection of premature drug delivery, as compared to the lipid-coated MBs. Another advantage of the polymeric MBs is that they can carry both hydrophilic and hydrophobic drugs, such as proteins and DNA plasmids. A commonly used polymeric shell is the biodegradable poly(lactide-co-glycolide) copolymer (PLGA) shell, consisting of a certain lactate and glycol concentration[37].

Drug loading of microbubbles

Different ways of drug loading have already been developed for different ways of drug delivery. In figure 2, these types of drug loading are demonstrated. The MBs depicted here are either lipid-coated (figure 2a trough d) or polymer-coated (Figure 2e through g). Hydrophilic macromolecules, like DNA and RNA, can be directly coupled to the outer lipid layer of the

MB (figure 2a). Amphilic molecules can be penetrated into the lipid monolayer (2b). Hydrophobic molecules can be incorporated in a thick layer of oil in the lipid shell (2c). They can alternatively be incorporated in secondary carriers, which in turn can bind to the outer layer of the MB (2d). Drugs can also be loaded into the shell of the MBs. A thick layer of biocompatible material, such as gelatin, can cover the capsule for protection against degradation (2e). Lastly, drugs can be loaded into smaller liposomes or NPs to form a bigger MB (2f and 2g)[38]. In this case, for instance, NPs can be loaded with drugs beforehand and directly be coupled to the surface of the MBs using a biotin-avidin-biotin bridging system. This means that both a lipid and a polymeric MB can be constructed[37].

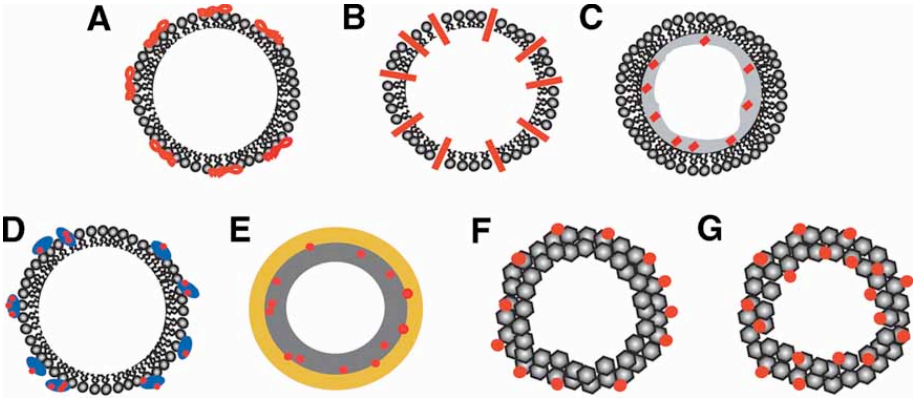

Figure 2. Different MB structures and drug loading methods (drug is displayed in red). A. Attachment to the outer shell surface, B. Intercalation between the two monolayer phospholipids, C. incorporation in a layer of oil, D. complexes with smaller particles (secondary carriers), E. Encapsulation in polymer layer (gray) and coating by biocompatible material (yellow). F. surface binding to protein shelled MBs, and G. entire volume loading of protein-shelled MBs (Adapted from Tinkov et al, 2009[38]).

Drug release after injection

Drug-loaded particles can be injected systemically or locally at site of treatment. MPs, for instance, have been used in animal models for local injection. MPs can be injected intramyocardially for treatment of CAD and intramuscularly for PAD treatment. Figure 3 describes a rat model in which a myocardial infarction was induced. Drug-loaded MPs were injected in the myocardium afterwards. This resulted in local drug delivery resulting in a small inflammatory tissue response, as indicated in the figure[39]. Cleland et al. used MPs for local delivery of rhVEGF in rats, which resulted in an angiogenic effect of local vessel sprouting in a dose-dependent manner[40]. In a mouse model for hind limb ischemia, bFGF was administered intramuscularly after femoral artery ligation. The controlled release of bFGF

improved blood perfusion in the ischemic limb dose-dependently, due to local formation of collateral vessels. In a mouse model for myocardial infarction, injection of MPs loaded with bFGF resulted in local controlled release of the drug as well. Angiogenesis was induced locally, which resulted in improvement of myocardial blood flow in the peri-infarcted area, as well as improvement of systolic and diastolic LV functions[41].

Using MBs is a way to achieve drug delivery at a higher dose at once, but with a slow release pattern locally. This type of drug delivery results in a less frequent drug administration. Furthermore, drugs can be administered systemically as well as locally[38].

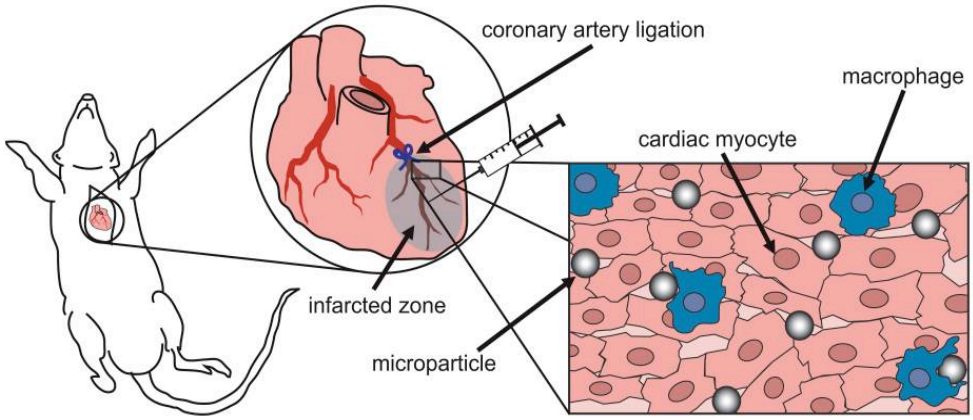

Figure 3.Permanent ligation of the left descending coronary artery created an infarcted zone. Microparticles were injected intramyocardially where they can release the encapsulated drug or gene (adapted from Sy et al, 2008[39]).

MBs can generally be loaded with drugs using two different methods. The drugs can be attached on the outside of the MB or they can be incorporated in the gas core of the MB[42]. Using the first method, the MBs can recognize targeted tissues by coating antibodies on the outside of the MB, as mentioned before. After binding of the MB, slow release of the drugs can take place locally, due to slow degradation of the MBs[37]. When the drug is loaded inside the gas core, ultrasound is needed for destruction of the MB. This will lead to local drug release from the MB[42]. Entrapment of the drug inside of the polymeric MB can be achieved by a number of ways. These include the formation of the water-in-oil emulsion with a water soluble protein and an organic solvent-borne polymer (emulsion method), the formation of a solid-in-oil suspension with a solid protein dispersed in a solvent-based polymer solution (suspension method), dissolving the protein in a solvent-based polymer solution (dissolution method), or by spray dying. But the most used method is the water-in-oil-in-water double emulsion technique (w/o/w)[35]. The rate of drug release is dependent on the conformation of the polymeric MB. Controlled release of the encapsulated drug can vary between 1 to even 3

months. Kinetics of drug release and stability of the MB itself is dependent on different factors, such as the polymer construct, the protein itself and its stability problems, and the formulation of the MB. Details are reviewed elsewhere[43].

Ultrasound can be used for destruction of the MBs for immediate local drug release. Diagnostic ultrasound normally operates in the frequency range of 1-10 MHz. Ultrasound administration can help with the penetration of the drugs through various tissues, it has direct effect on the membrane permeability of the targeted tissue and it can change chemical properties of the drug itself[44]. In figure 4, a schematic overview of MB destruction by ultrasound is depicted. When drug-loaded MBs reach the site of desired drug administration ultrasound is applied locally. MBs are destroyed locally resulting in local drug release for a direct effect on the surrounding tissues[45].

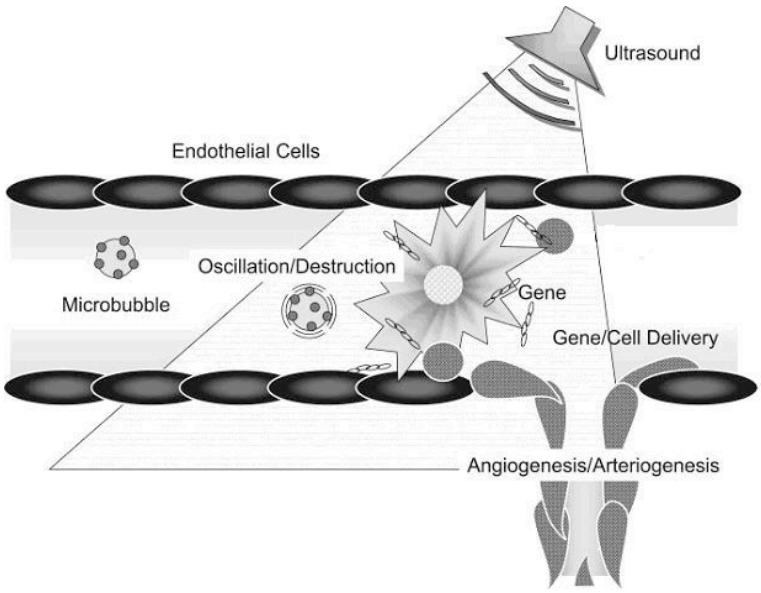

Figure 4. At the site of desired drug administration, ultrasound is applied. Microbubbles reaching this site will be destructed by ultrasound, which results in a local release of drugs for a direct effect on the tissue (adapted from Takahashi et al, 2007[45]).

Behaviour of the two MB subtypes is quite different after ultrasound exposure. Lipid-coated MBs are only destroyed at high ultrasound intensity. This is different for the polymer-coated MBs. At a low intensity, the stiff polymeric shell will not oscillate actively. When the ultrasound intensity increases, the shell will show cracks. Through these cracks the encapsulated gases will escape, together with the drugs [37]. This behaviour is illustrated in figure 5.

Dependent on the requested rate of drug release, ultrasound can be applied for immediate and local drug release or MBs can be kept in their original state for slow drug release. During slow drug release, a sustained drug level will be present at the requested site. In the case of gene transfection, ultrasound can contribute to the rate of transfection by locally destroying the cells.

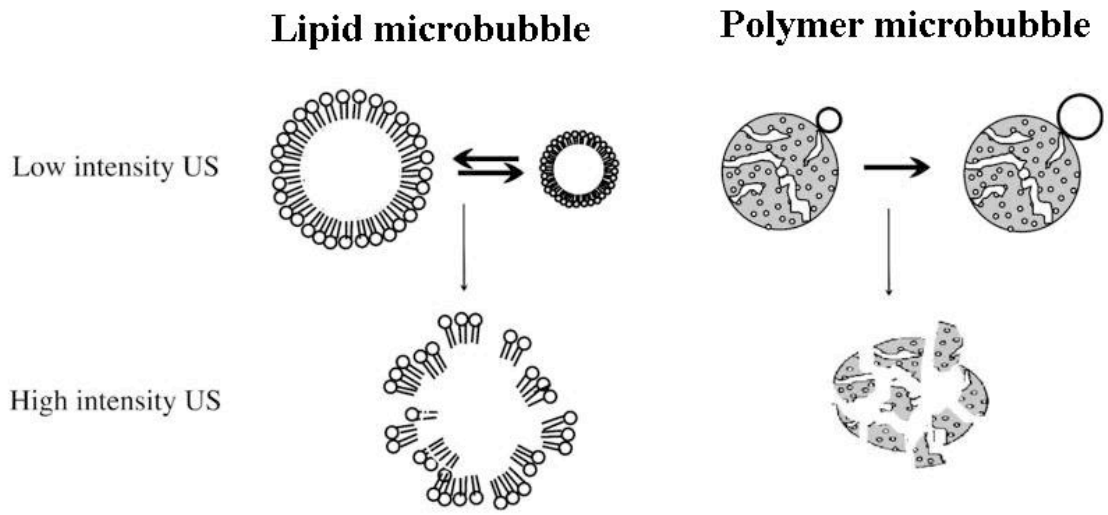

Figure 5. Schematic overview of lipid and polymer microbubble behaviour after ultrasound appliance in low and high intensity. (Adapted from Hernot & Klibanov, 2008[37])

Microbubble and ultrasound use in animal studies

Different animal studies have shown very promising results after administration of drugs via MBs. Using MBs for gene transfection in a mouse heart resulted in a 10-fold increased expression compared to all controls[46]. MBs have been injected in different animal models for myocardial infarction. MBs contained, for instance, DNA plasmids for p53 or VEGF. Drug delivery via MBs resulted in high expression levels of the genes locally. This gene transfection resulted in an increase of collateral growth as well. In some cases, it even resulted in lower infarct sizes and better LV contractile function[34]. In a mouse model for hind limb ischemia, VEGF administration by MB destruction using ultrasound resulted in therapeutic arteriogenesis[5]. In a similar mouse model, MBs containing smaller NPs loaded with bFGF were injected intravascularly and the ischemic hind limb was exposed to ultrasound. This targeted delivery of bFGF from the NPs in the saphenous artery also led to therapeutic arteriogenesis after two weeks[22]. The experimental setup of this study is depicted in figure 6.

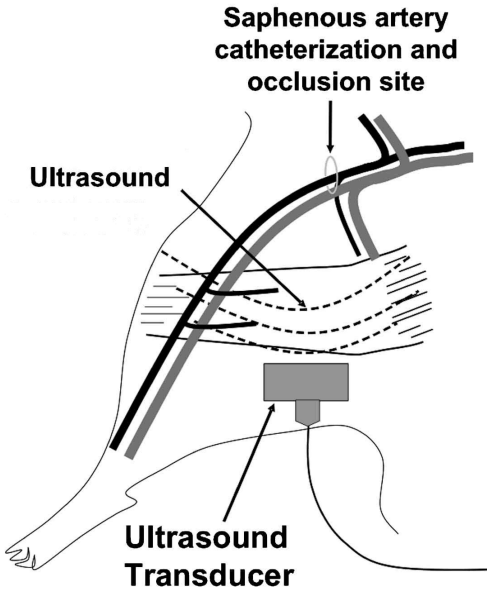

Figure 6. Schematic illustration of a mouse hind limb model including intravascular administration and local delivery of ultrasound (adapted from Chapell et al, 2008[22]).

Advantages and disadvantages of drug delivery systems

Direct administration of free drugs to enhance collateral growth can lead to high local concentrations of the drugs. However, these high concentrations of drugs can lead to increased risks for local edema and vascular injury, which can lead to atherosclerotic development. Therefore, controlled drug delivery would be more favourable using drug delivery systems[47].

Drug delivery systems have different advantages. These include extended release of the drug for days, weeks, or even months at a sustained level, and ease of administration of the drug. Also, repetition of drug delivery can easily be reduced. Disadvantages have also been reported in the past as well. Drug loading can be accompanied by negative side effects on protein stability during preparation and storage of the drug. To avoid destabilization, stabilizers can be added to the system, such as carrier proteins or surfactants[36].

Ultrasound administration itself can also result in negative side effects for the surrounding tissues. These effects were already shown in *in vitro* and *in vivo* experiments. Ultrasound use can result in hemolysis, capillary ruptures, petechial hemorrhages, inflammation due to injection of foreign material or thermal effects[37]. Cardiac side effects of ultrasound use include sudden contractions of the heart muscle. Furthermore, hemorrhages are also observed in the heart[34].

Future perspectives

Drug delivery systems are useful tools for local drug delivery for the enhancement of collateral growth. These systems have major clinical potential for patients suffering from CAD or PAD and for whom current therapies do not seem to work sufficiently. It has been shown in the past that administration of free drugs, locally or systemically, may lead to harmful side effects[37]. Encapsulation of drugs in carriers can avoid this and leads to improved local drug delivery with its therapeutic effects on collateral growth.

A drug delivery system needs to be organ specific and suitable for sustained release, when requested[35]. Drug delivery can either be achieved by protein delivery or gene delivery for cell transfection[34,36]. Several different carriers have been developed already. Drugs can be loaded in or attached to the outside of a carrier, such as nano- and microparticles. These carriers are small enough to pass through the lungs and move freely throughout the vasculature without causing an obstruction and without being obstructed[37]. One type of microparticle is the MB, this particular microparticle has frequently been tested in animal models. Different constructs can load either hydrophobic or hydrophilic drugs. Gene constructs have been used in MBs already, in either a viral or non-viral vector. Viral vectors are very effective in gene delivery, but can evoke an immune reaction and are limited in gene carrying capacity[48]. Non-viral vectors are more frequently used in animal models and have the major advantage that they do not evoke an immune reaction after injection[49].

For future MB use, MBs need to undergo further development to resolve current problems in its use. Ultrasound use for destruction of the MB leads to immediate release of the encapsulated drug. As shown in figure 5, this can be achieved using low or high ultrasound intensity. Gene constructs benefit enormously from local ultrasound administration for local cell destruction for better transfection[37]. However, when slow drug release is requested, the use of low ultrasound intensities is more favourable. The speed of shell destruction is not only dependent on ultrasound intensity, but shell composition can contribute to this phenomenon as well. Already, it has been shown that lipid-coated MBs release their drugs at a much higher speed, because the shell is destructed almost entirely after ultrasound administration[37]. In the case of polymer-coated MBs, a lower ultrasound intensity can only crack the shell open and a slower release of the drug can be achieved. By changing the composition and thickness of the polymer shell the speed of drug release can be influenced. When a slower release of a drug is requested, a thicker and more stable polymeric shell can be used.

Also, changing the composition of the polymeric shells can be helpful in the delivery of multiple drugs using one carrier. For example, a carrier could be constructed consisting of

different layers filled with different drugs. The different drugs can then be released at a slow rate, following each other through slow erosion of each polymer layer. No investigations have been done yet on constructions containing more than one drug. Using these multi-layered constructions, ultrasound at low intensity could also be used to achieve local drug release. Using ultrasound at different points in time, all layers can be cracked open following each other. This results in the release of different drugs following each other in time. All drugs can be released at any requested point in time during treatment. For sustained release of one drug, all layers can be filled with the same drug. This situation can imitate repetitive local drug injections. A point of discussion is the amount of layers used in one carrier. The MBs still need to be small enough for passage to avoid unwanted obstruction in the vasculature. Also, multilayered MBs need to stay stable over a longer period of time to avoid unwanted drug release in between different ultrasound administrations.

Furthermore, when two different types of drugs need to be incorporated into one carrier, the use of two gas cores can be a solution. Preparation of two gas cores containing different drugs helps to stabilize both drugs, because contact between these two drugs is avoided. In this way, drugs can be loaded together into one carrier and also be released at the same time.

Another option, what has already been investigated in the past, is the binding of small drug-loaded NPs on the outer layer of MBs, either lipid- or polymer–coated. In this way, smaller drug–loaded particles are attached to bigger carriers for local drug release[37]. Alternatively, these NPs can be loaded into the gas core of an MB and be released after erosion or ultrasound destruction of the MB. In both ways, hydrophilic and hydrophobic drugs can be combined into one carrier.

A disadvantage of drug delivery systems could be the eventual clearance of the particles by the body, for example the immune system. Especially, when using multi-layered MBs, it would be desirable to achieve survival in the body for a prolonged period of time.

Therefore, the chemical composition of the carriers needs more attention[44]. Depending on the goal of the treatment, the MB composition needs to be modified for optimal use. These changes can be helpful for either slow and sustained drug release or immediate drug release by ultrasound destruction.

Construction of these different types of MBs is still in the developing phase. Newly developed carriers need to be tested in animal models first before they can be tested in clinical trials. When focusing on stimulation of collateral growth itself, drug choice is another issue. In the case of multi-layered or multi-loaded carriers as described above, it is important to investigate which drug combinations are most effective in the stimulation of collateral growth.

To summarize, drug delivery systems can be useful tools in promoting collateral growth. However, for clinical applications in the near future, more research should be conducted focusing on the construction of the particle, the way of drug incorporation and the chemical compositions of the different shells.

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