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(54) **Title**: COMPOSITION COMPRISING AN ENCAPSULATED ANTAGOMIR

(57) **Abstract**: The invention relates to a composition comprising an effective amount of at least one inhibitor of a miRNA involved in the angiogenesis, or a precursor thereof, wherein said inhibitor is microencapsulated into polymeric biodegradable and biocompatible microspheres. The invention also relates to the use of the said composition for preventing or treating cardiac disorders, including cardiac disorders caused by ischemy.

"Composition comprising an encapsulated antagomir"

Field of the invention

The present invention relates to the field of pharmaceutical formulations aimed at preventing or treating cardiac disorders, including cardiac ischemic disorders.

Background of the invention

Acute myocardial infarction (AMI), also referred as myocardial infarction and commonly known as a heart attack, represents a major health risk in most industrialized nations throughout the world and remains a leading cause of morbidity and mortality worldwide.

Generally, AMI is caused by a sudden and sustained lack of blood flow to the heart tissue, which is usually the result of a narrowing or occlusion of a coronary artery. Without adequate blood supply, the tissue becomes ischemic, leading to the death of cardiomyocytes (heart muscle cells) and vascular structures.

There have been many advances in the treatment of AMI in recent decades, primarily in relation to coronary reperfusion in conjunction with pharmacological therapy. Reperfusion therapy has succeeded in changing the natural progression of AMI by reducing the infarcted area and improving short-term and long-term morbidity and mortality. However, there are substantial limitations to the use of the two reperfusion strategies currently available: fibrinolysis results in a low degree of coronary permeability and primary angioplasty cannot be applied frequently during the initial hours of evolution of the AMI. Furthermore, in some catheterized patients, the "no reflow" phenomenon or absence of microvascular reperfusion despite normal epicardial flow occurs, which results in adverse functional outcomes. This means that reperfusion therapy has not prevented the occurrence of deleterious remodelling of the myocardium, a complex intrinsic reparative process of collagen scar formation resulting in ventricular dilatation, contractile dysfunction and subsequent heart failure. Its occurrence, in approximately 30% of AMIs, has been mainly associated to higher infarction size, microvascular obstruction and unfavourable repair reactions still poorly understood.

Since reparative fibrosis and functional recovery of ischemic tissues is dependent on establishing networks that supply oxygenated blood, efforts have been made to improve vascular bed by inducing neoangiogenesis in the healing area for achieving direct conversion of injured areas to functional tissue *in situ*.

Therapeutic induction of angiogenesis could attenuate the occurrence of this phenomenon. Notwithstanding, the results of several years of clinical trials with intravenous proangiogenic factors have been unsatisfactory. Without being linked by a theory, it is considered that one of the reasons accounting for this failure may be that the intravenous route may not be able to reach and maintain an effective concentration of drug in the target tissue to promote and maintain a functional vascular network under severely compromised heart conditions.

In order to address this problem and prevent post-AMI cardiac insufficiency, research on the possibility of regenerating cardiac muscle and vessels was started within the last decade. Initial studies administrating pluripotent progenitor cells and infusing vascular growth factors showed promising results but the translation to the clinical setting showed inability of these therapies to regenerate neovasculature in an adequate manner for enabling destroyed cardiac muscle to recover.

At this day, the angiogenesis and repopulation of injured heart with cell therapy as well as specific drugs and resynchronization therapy can slow down the progression of this phenomenon but prevention of its occurrence remains a doubting challenge for cardiac medicine. Therefore, novel treatment strategies preventing adverse left ventricular remodelling are required.

Summary of the invention

The present invention relates to the treatment of cardiac, preferably ischemic disorders by administering a composition that reverses or prevents ventricular remodelling by modulating the activity or expression of microRNAs. More particularly, the invention provides a composition comprising an inhibitor of a microRNA involved in angiogenesis, said inhibitor being preferably incorporated into a new delivery vehicle capable of releasing the inhibitor locally to the ischemic target tissue. In addition, the invention provides a method of reversing or preventing ventricular remodelling and kits useful for said method.

This invention relates to a composition comprising an effective amount of at least one inhibitor of a miRNA involved in the angiogenesis, or a precursor thereof, wherein said inhibitor is microencapsulated into polymeric biodegradable and biocompatible microspheres.

In some embodiments of the composition described above, the said miRNA is selected in the family comprising miR-92 (including miR-92a-1, miR-92a-2 and miR-92b),

miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b).

In some embodiments of the composition described above, the said inhibitor of a miRNA is an oligonucleotide of 8-49 nucleotides in length having a sequence targeted to the said miRNA, or a precursor thereof. In certain embodiments, the said oligonucleotide is an antisense oligonucleotide that is at least partially complementary to the sequence of the target miRNA, or a precursor thereof. The said antisense oligonucleotide may be selected from the group consisting of a ribonucleotide, a deoxyribonucleotide, a small RNA, an antagomir, a LNA, a CDNA, a PNA, a morpholino oligonucleotide or a combination thereof.

In some embodiments of the composition described above, the said inhibitor of a miRNA consists of an antagomir, and preferably consists of an antagomir comprising a nucleotide sequence comprising at least 16 contiguous nucleotides complementary to the nucleotides of a sequence selected from the group consisting of SEQ ID No. 1 to 58 described herein.

In some embodiments of the composition described above, the said inhibitor of a miRNA consists of an antagomir comprising the sequence SEQ ID No. 59 or 60 and modifications excluding base substitutions thereof, and fragments consisting of subsequences of SEQ ID NO: 59 or 60 of at least 8 contiguous nucleotides thereof.

In some embodiments of the composition described above, the said microspheres are presenting a diameter which does not exceed 25 μm, which encompasses microspheres having a diameter comprised between 5 and 20 μm, preferentially between 5 and 15 μm.

In some embodiments of the composition described above, the said microspheres are made of a polymer consisting of poly-d,l-lactide (PLA), the said polymer being optionally blended with one or more other polymers.

This invention also relates to a method for reversing or preventing ventricular remodelling in a subject in need thereof comprising administering to said subject an effective amount of a composition as described above.

This invention also pertains to a population of biodegradable and biocompatible microspheres for use in the treatment or prevention of myocardial infarction, wherein said microspheres:

- have an average diameter comprised between 5 and 20 µm, preferentially between 5 and 15 µm;
- are made of poly-d,l-lactide-co-glycolide (PLGA) ; poly-d,l-lactide (PLA) or a blend thereof;
- are incorporating from 1% to 15% w/w of a therapeutic agent capable of preventing ventricular remodelling,

wherein said therapeutic agent consists of an inhibitor of a miRNA selected from the group consisting of miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b) and more preferentially miR-92a, or a precursor thereof.

Description of the figures

Figure 1 illustrates the morphology of antagomir-92a – PLGA microspheres determined by scanning electron microscopy;

Figure 2 illustrates the size distribution of antagomir-92a – PLGA microspheres determined by laser light scattering;

Figure 3 illustrates effects in hemodynamic and left ventricular contractibility by repeated injections of microspheres until 120mg;

Figure 4 illustrates effects in hemodynamic and left ventricular contractibility by repeated injections of microspheres until 240mg;

Figure 5 illustrates the protocol of the study of the molecular effect of a single intracoronary injection of microspheres according to the invention;

Figure 6 illustrates the miR-92a inhibition specificity by the microspheres according to the invention;

Figure 7 illustrates the encapsulated antagomir-92a induces angiogenesis in infarcted tissue. Vascular density in infarcted zone, calculated dividing the total number of vessels by the total infarcted area. N=20. * $P< 0.01$;

Figure 8: Indirect Measurement. Basal and minimal microvascular resistance index measured one month after AMI, in the infarct related artery of minipigs treated with encapsulated antagomir-92a, placebo or saline. (**a**) Basal microvascular resistance index (MRI) was calculated by multiplying pressure (mmHg) assessed with a pressure wire in

positioned in the apical LAD (Pd) and coronary flow (ml/min) quantified by a sensor positioned in the distal segment of LAD. Minimal microvascular resistance index (MRI_{hyp}) was calculated with the same parameters measured in maximal hyperemia achieved with intravenous infusion of 140 micro/Kg/min of adenosine administered through the femoral vein 12F sheath. n= 12 * p<0.01 ** p=0.05 (**b**) Correlation between the total number of vessels in the necrotic area and MRI. R^2 0.41, p=0.02, n=12. (**c**) Correlation between the total number of vessels in the necrotic area and minimal MRI. R^2 0.27, p=0.08, n=12;

Figure 9: Direct Measurement. Baseline and true microcirculatory resistance measured one month after AMI in the infarct-related artery of minipigs treated and non-treated with encapsulated antagomir-92a. (**a**) Baseline microcirculatory resistance (baseline MR) was calculated by dividing pressure (mmHg) assessed with a pressure wire positioned in the apical LAD (Pd) by coronary flow (ml/min) quantified by a sensor positioned in the distal segment of LAD. The baseline MR was significantly lower in the treated group compared with controls (7.47±1.33 vs 19.62±2.98, p=0.005). n=13. True microcirculatory resistance (TMR (hyp)) was calculated measuring the same parameters during maximal hyperemia achieved with intravenous infusion of 140 micro/kg/min of adenosine administered through the femoral vein 12F sheath. Significantly lower TMR (hyp) was observed in the treated group compared to controls (5.0±1.15 vs 14.49±2.4, p= 0.006). n= 13 (**b**) Correlation between the vascular density in all necrotic area and baseline MR. R^2=0.35, *P*=0.033, n=13. (**c**) Correlation between the vascular density in all necrotic area and TMR (hyp). R^2=0.31, *P*=0.047. n=13.

Figure 10: One month after AMI, the presence of septoapical diskynesia was evaluated by an echocardiographer blind to the allocated treatment. The percentage of animals with septoapicaldiskynesia in treated and non-treated animals is shown (83.3% vs 16.7%, p=0.03). n=20.

Figure 11: evaluation of the effects of encapsulated antagomir 92a and non encapsulated antagomir 92a on the expression of miR92a *in vitro*.

Figure 12: Effect of encapsulated antagomirs 17 and 20a on their respective miRNAs

Detailed Description of the invention

Some definitions are given hereunder that are relevant as regards the description of the whole embodiments that are encompassed by the present invention.

MicroRNAs (miRs) are small, noncoding RNAs that are emerging as crucial regulators of biological processes.

"MicroRNA", "miRNA" or "miR" means a non coding RNA of about 18 to about 25 nucleotides in length. These miRs could originate from multiple origins including: an individual gene encoding for a miRNA, from introns of protein coding gene, or from poly-cistronic transcript that often encode multiple, closely related microRNAs.

Current knowledge shows that miRNAs are transcribed by RNA polymerase II (pol II) or RNA polymerase III (pol III) and arise from initial transcripts, termed primary miRNA transcripts (pri-miRNAs), that are generally several thousand bases long. Pri-miRNAs are processed in the nucleus by the RNase Drosha into about 70 to 100-nucleotide hairpin-shaped precursors (pre-miRNAs). Following transport to the cytoplasm, the hairpin pre-miRNA is further processed by Dicer to produce a double-stranded microRNA; one of the strands, the so called mature microRNA, (sometimes both strands can be used) is then incorporated into the RNA-induced silencing complex (RISC), where it associates with its target mRNAs by base-pair complementarity. In the relatively rare cases in which a miRNA base pairs perfectly with a messenger RNA (mRNA) target, it promotes mRNA degradation. More commonly, microRNAs form imperfect heteroduplexes with target mRNAs, affecting either mRNA stability or inhibiting mRNA translation.

"Stem-loop sequence" means a RNA having a hairpin structure and containing a mature microRNA sequence. Pre-miRNA sequences and stem-loop sequences may overlap. Examples of stem-loop sequences are found in the microRNA database known as miRBase.

"microRNA precursor" means a transcript that originates from a genomic DNA and that comprises a non-coding, structured RNA comprising one or more microRNA sequences. For example, in certain embodiments a microRNA precursor is a pre-miRNA. In certain embodiments, a microRNA precursor is a pri-miRNA.

The following specification will follow the standard nomenclature system with the uncapitalized "mir-X" refers to the pre-miRNA, while a capitalized "miR-X" refers to the mature form. When two mature microRNAs originate from opposite arms of the same pre-miRNA, they are denoted with a -3p or -5p suffix. When relative expression levels are known, an asterisk following the name indicates a microRNA expressed at low levels relative to the microRNA in the opposite arm of a hairpin.

In the following specification, unless otherwise specified, the use of the expression miR-X refers to the mature miRNA including both forms -3p and -5p, if any.

For the avoidance of doubt, in the present specification, the expressions microRNA, miRNA and miR designate the same product.

In the context of the present invention, it is an objective to modulate angiogenesis or angiogenic processes with, as a consequence, a particular focus on microRNA involved in angiogenesis. It is thus an objective of the invention to modulate at least one microRNA belonging to a family of microRNAs involved in the modulation of the angiogenesis.

By the expression "microRNA family", it is intended a group of microRNAs with a related function consisting of the modulation of angiogenesis or angiogenic processes. More particularly, without limitation, said microRNAs are selected in the group consisting of microRNAs presenting an antiangiogenic activity.

More particularly, without limitation, such microRNA are selected in the group consisting of miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b), or precursors thereof. As it will be obvious for the person skilled in the art, any miRNA having modulating, preferentially antagonist, properties on angiogenesis should be considered as part of this microRNA family.

For the avoidance of doubt, the expression microRNA in the following specification, unless otherwise indicated, shall refer to the mature or processed RNA after it has been cleaved from its precursor. For the non mature forms, the expression "precursors" or "microRNA precursors" will be used.

The following table 1 regroups the different sequences of the microRNA precursors encompassed by the present specification.

SEQ ID NO:	Name	Sequence
1	mir-92a-1	CUUUCUACACAGGUUGGGAUCGGUUGCAAUGCUGUGUUUCUGUAUGGUAUUG CACUUGUCCCGGCCUGUUGAGUUUGG
2	mir-92a-2	UCAUCCCUGGGUGGGGAUUUGUUGCAUUACUUGUGUUCUAUAUAAAGUAUUG CACUUGUCCCGGCCUGUGGAAGA
3	mir-92b	CGGGCCCCGGGCGGGCGGGAGGGACGGGACGCGGUGCAGUGUUGUUUUUUCC CCCGCCAAUAUUGCACUCGUCCCGGCCUCCGGCCCCCCCGGCCC
4	mir-17	GUCAGAAUAAUGUCAAAGUGCUUACAGUGCAGGUAGUGAUAUGUGCAUCUAC UGCAGUGAAGGCACUUGUAGCAUUAUGGUGAC
5	mir-503	UGCCCUAGCAGCGGGAACAGUUCUGCAGUGAGCGAUCGGUGCUCUGGGGUAU UGUUUCCGCUGCCAGGGUA
6	mir-16-1	GUCAGCAGUGCCUUAGCAGCACGUAAAUAUUGGCGUUAAGAUUCUAAAAUUA UCUCCAGUAUUAACUGUGCUGCUGAAGUAAGGUUGAC
7	mir-16-2	GUUCCACUCUAGCAGCACGUAAAUAUUGGCGUAGUGAAAUAUAUAUUAAACA CCAAUAUUACUGUGCUGCUUUAGUGUGAC
8	mir-374a	UACAUCGGCCAUUAUAAUACAACCUGAUAAGUGUUAUAGCACUUAUCAGAUU GUAUUGUAAUUGUCUGUGUA
9	mir-374b	ACUCGGAUGGAUAUAAUACAACCUGCUAAGUGUCCUAGCACUUAGCAGGUUG UAUUAUCAUUGUCCGUGUCU
10	mir-374c	ACACGGACAAUGAUAAUACAACCUGCUAAGUGCUAGGACACUUAGCAGGUUG UAUUAUAUCCAUCCGAGU
11	mir-24-1	CUCCGGUGCCUACUGAGCUGAUAUCAGUUCUCAUUUUACACACUGGCUCAGU UCAGCAGGAACAGGAG
12	mir-24-2	CUCUGCCUCCCGUGCCUACUGAGCUGAAACACAGUUGGUUUGUGUACACUGG CUCAGUUCAGCAGGAACAGGG
13	mir-483	GAGGGGGAAGACGGGAGGAAAGAAGGGAGUGGUUCCAUCACGCCUCCUCACU CCUCUCCUCCCGUCUUCUCCUCUC
14	mir-34a	GGCCAGCUGUGAGUGUUUCUUUGGCAGUGUCUUAGCUGGUUGUUGUGAGCAA UAGUAAGGAAGCAAUCAGCAAGUAUACUGCCCUAGAAGUGCUGCACGUUGUG GGGCCC
15	mir-34b	GUGCUCGGUUUGUAGGCAGUGUCAUUAGCUGAUUGUACUGUGGUGGUUACAA UCACUAACUCCACUGCCAUCAAAACAAGGCAC
16	mir-34c	AGUCUAGUUACUAGGCAGUGUAGUUAGCUGAUUGCUAAUAGUACCAAUCACU AACCACACGGCCAGGUAAAAAGAUU
17	mir-20a	GUAGCACUAAAGUGCUUAUAGUGCAGGUAGUGUUUAGUUAUCUACUGCAUUA UGAGCACUUAAAGUACUGC
18	mir-20b	AGUACCAAAGUGCUCAUAGUGCAGGUAGUUUUGGCAUGACUCUACUGUAGUA UGGGCACUUCCAGUACU
19	mir-15a	CCUUGGAGUAAAGUAGCAGCACAUAAUGGUUUGUGGAUUUUGAAAAGGUGCA GGCCAUAUUGUGCUGCCUCAAAAAUACAAGG
20	mir-15b	UUGAGGCCUUAAAGUACUGUAGCAGCACAUCAUGGUUUACAUGCUACAGUCA AGAUGCGAAUCAUUAUUUGCUGCUCUAGAAAUUUAAGGAAAUUCAU

Table 1

The following table 2 indicates, for each microRNA, the sequences of the microRNA and the corresponding residues into the corresponding precursor sequences (see table 1).

SEQ ID NO:	Name	Sequence	Residues from precursor
21	miR-92a-3p	uauugcacuugucccggccugu	48-69
22	miR-92a-1-5p	agguugggaucgguugcaaugcu	11-33
23	miR-92a-2-5p	gggugggggauuuguugcauuac	9-30
24	miR-92b-3p	uauugcacucgucccggccucc	61-82
25	miR-92b-5p	agggacgggacgcggugcagug	20-41
26	miR-17-3p	acugcagugaaggcacuuguag	51-72
27	miR-17-5p	caaagugcuuacagugcagguag	14-36
28	miR-503-3p	gggguauuguuuccgcugccagg	46-68
29	miR-503-5p	uagcagcgggaacaguucugcag	6-28
30	miR-16-1-3p	ccaguauuaacugugcugcuga	56-77
31	miR-16-2-3p	ccaauauuacugugcugcuuua	53-74
32	miR-16-5p	uagcagcacguaaauauuggcg	14-35 (for miR-16-1) or 10-31 (for miR-16-2)
33	miR-374a-3p	cuuaucagauuguauuguaauu	42-63
34	miR-374a-5p	uuauaauacaaccugauaagug	12-33
35	miR-374b-3p	cuuagcagguuguauuaucauu	41-62
36	miR-374-5p	auauaauacaaccugcuaagug	11-32
37	miR-374c-3p	cacuuagcagguuguauuauau	39-60
38	miR-374c-5p	auaauacaaccugcuaagugcu	13-34
39	miR-24-1-3p	uggcucaguucagcaggaacag	44-65
40	miR-24-1-5p	ugccuacugagcugauaucagu	7-28
41	miR-24-2-3p	uggcucaguucagcaggaacag	50-71
42	miR-24-2-5p	ugccuacugagcugaaacacag	13-34
43	miR-483-3p	ucacuccucuccucccgucuu	48-68
44	miR-483-5p	aagacgggaggaaagaagggag	8-29
45	miR-34a-3p	caaucagcaaguauacugcccu	64-85
46	miR-34a-5p	uggcagugucuuagcuggguugu	22-43
47	miR-34b-3p	caaucacuaacuccacugccau	50-71
48	miR-34b-5p	uaggcagugucauuagcugauug	13-35

49	miR-34c-3p	aaucacuaaccacacggccagg	46-67
50	miR-34c-5p	aggcaguguaguuagcugauugc	13-35
51	miR-20a-3p	acugcauuaugagcacuuaaag	44-65
52	miR-20a-5p	uaaagugcuuauagugcagguag	8-30
53	miR-20b-3p	acuguaguaugggcacuuccag	44-65
54	miR-20b-5p	caaagugcucauagugcagguag	6-28
55	miR-15a-3p	caggccauauugugcugccuca	51-72
56	miR-15a-5p	uagcagcacauaaugguuugug	14-35
57	miR-15b-3p	cgaaucauuauuugcugcucua	58-79
58	miR-15b-5p	uagcagcacaucaugguuuaca	20-41

Table 2

Unless otherwise indicated, precursor and microRNA sequences referred to in the application are human sequences. Nevertheless, in some cases, microRNA human sequences are homologous to microRNA sequences from other species.

As an example, it can be mentioned here that the human miR-92a is homologous to miRs from other species. More particularly, the human miR-92a is homologous to miRs from dme (*Drosophila melanogaster*), mmu (*Mus musculus*), rno (*Rattus norvegicus*), dps (*Drosophila pseudoobscura*), aga (*Anopheles gambiae*), dre (*Danio rerio*), mml (*Macaca mulatta*), xtr (*Xenopus tropicalis*), ame (*Apis mellifera*), odi (*Oikopleura dioica*), cin (*Ciona intestinalis*), csa (*Ciona savignyi*), cfa (*Canis familiaris*) and pig or ssc (*Sus scrofa*).

Based on its general knowledge, the person skilled in the art will find easily the homology between other human microRNA sequences and microRNA sequences from other species.

Nucleotide sequences of mature microRNAs and their corresponding stem-loop sequences described herein are the sequences found in miRBase, an online searchable database of microRNA sequences and annotation. Entries in the miRBase Sequence database represent a predicted hairpin portion of a microRNA transcript (the stem-loop), with information on the location and sequence of the mature microRNA sequence. The microRNA stem-loop sequences in the database are not strictly precursor miRNAs (pre-

miRNAs), and may in some instances include the pre-miRNA and some flanking sequence from the presumed primary transcript.

It is thus an objective of the present invention to provide compositions for the treatment or prevention of ventricular remodelling after AMI comprising an inhibitor of microRNA, or a combination of inhibitors of several microRNAs, involved in different reactions governing the physiology of angiogenesis, or precursors thereof.

However, in most of the published studies, the intravenous route is used for administering microRNA inhibitors in animals. The manipulation of microRNA involved in the regulation of vascular genes expression represents a novel therapeutic target in ischaemic disease. Within the biomedical sector, there has been an exponential increase in research on the administration of RNA modulators especially designed to inhibit a particular RNA sequence.

Dimmeler et al have shown improvement in contractility and recovery of left ventricular function after miR-92a inhibition by specific microRNA inhibitor administered systemically. Since the polycistronic microRNA 17-92a cluster has been linked to tumorigenesis and because of microRNAs cellular type ubiquity, intravenous administration in repetitive injections of miR or anti-miRs raises concerns about safety.

Owing to the fact that microRNAs control complex processes and are present in various cellular pathways, it is highly probable that they also trigger significant side effects. An issue, among other ones, at present is that treatments with miRNA inhibitors are not selective.

In addition, given the ubiquitousness and low organ specificity of these molecules, systemic administration could lead to exercise regulatory functions in tissues where these miRNAs have different cell-specific functions or where are not normally expressed. This erroneous regulation would likely lead to triggering side effects. Among the potential risks, tumorigenesis associated with microRNA manipulation remains one of the primary concerns when applying this therapy to human pathology. Moreover, in order to obtain an adequate sustained concentration in the target cells, microRNA inhibitors must be repeatedly injected at very high doses. Although experimentation on small animals in controlled conditions carries minor limitations, the transposition to human beings presupposes obstacles to biosafety as well as logistical and economic hindrances.

In order to solve these problems and enable this new therapeutic approach to be transferred to patients, it is there considered to generate a release vehicle for transporting microRNA inhibitors and releasing them directly onto the target organ. An appropriate

vehicle would direct the microRNA inhibitor straight to the diseased tissue thereby enabling a reduction in dosage, so as to avoid the administration of repeated injections and minimise potentially undesirable biological effects on other organs.

The objective is therefore to improve selectivity in order to avoid the side effects of these treatments by means of releasing the microRNA inhibitors only in target tissue. This issue is solved, according to the invention, by microencapsulating the microRNA inhibitors into biodegradable and biocompatible microspheres.

It is thus an objective of the present invention to provide compositions for the treatment or prevention of ventricular remodelling after AMI comprising at least one microencapsulated inhibitor of a microRNA belonging to the microRNA family related to the modulation of angiogenesis, which microRNA family comprises, without limitation, the miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b), or a precursor thereof.

By microencapsulating microRNA inhibitors in microspheres, intra-arterial administration after transluminal angioplasty is facilitated, so that the microspheres are delivered and retained only in the microvessels, also referred to as capillaries, of the damaged area; in this way the encapsulated microRNA inhibitors can be released locally.

Intracoronary injection through the culprit artery of AMI enables retention of the microspheres in the coronary microcirculation and sustained release of the microRNA inhibitors directly in the target ischemic tissue. The microRNA inhibitors induce neoangiogenesis which enhances functional recovery of the contractility of the damaged tissue as well as favourable post-infarction remodelling.

Unexpectedly, it is shown herein that, when it is microencapsulated into appropriate microspheres, a microRNA inhibitor and especially an inhibitor of a microRNA belonging to the microRNA family related to the modulation of angiogenesis, the said microRNA is successfully released to the damaged area of the myocardium so as to block the biological activity of the target microRNA. As shown in the examples herein, the administration, by selective intracoronary route, of a given microRNA inhibitor, i.e. as non limitative example an inhibitor of miR-92a, that is microencapsulated into polymeric biodegradable and biocompatible microspheres to individuals having undergone a myocardial infarction leads to myocardial functional recovery.

Therefore, microspheres retained in microcirculation allow sustained release of the microRNA inhibitor directly in the target ischemic tissue. The sustained effect of microRNA inhibitors (also known as down-regulation) of targeted microRNA resulted in a significant vessel growth and suppression of adverse remodeling in the healing area one month after injury.

As shown in the examples herein, it has been demonstrated that the occurrence of adverse ventricular remodelling can be prevented by inducing vasculogenesis through local and sustained inhibition of a microRNA belonging to the microRNA family related to the modulation of angiogenesis by encapsulated appropriate microRNA inhibitor administered in the infarcted related artery. This represents a new delivery method that will facilitate the next safety translation of gene modulation therapy to patients that suffer an acute myocardial infarction.

These results clearly show that a composition comprising an inhibitor of microRNA belonging to the microRNA family related to the modulation of angiogenesis, such as miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b), or a precursor thereof, as disclosed herein allows an effective local release of the said inhibitor in therapeutically effective amounts and at a therapeutically effective release rate.

In a first aspect, the invention relates to a composition comprising an effective amount of at least one inhibitor of a microRNA involved in the angiogenesis, or a precursor thereof, wherein said inhibitor is microencapsulated into polymeric biodegradable and biocompatible microspheres.

In another aspect, the invention relates to a composition comprising an effective amount of at least one inhibitor of a microRNA selected in the family comprising miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b), or a precursor thereof, wherein said inhibitor is microencapsulated into polymeric biodegradable and biocompatible microspheres.

In still another aspect of the invention, the invention relates to a composition comprising an effective amount of at least one inhibitor of miR-92a, or a precursor thereof, wherein said inhibitor is microencapsulated into polymeric biodegradable and biocompatible microspheres.

By the expression "microspheres", it must be understood spherical particles sized from 1μm to few hundred μm. The expression "microparticles" includes both "microspheres" and "microcapsules". In the present specification, the expression "microspheres" is used but it must be understood that, when the substitution with a "microcapsule" is possible and of interest according to the man skilled in the art, the use of "microspheres" and "microcapsules" is equivalent.

By the expression "encapsulated" or "microencapsulated", it must be understood enclosed or embedded in particles for protection or for modified release.

In other words, the invention relates to a pharmaceutical composition comprising an effective amount of at least one inhibitor of a microRNA involved in angiogenesis, said inhibitor of a microRNA being preferably selected from the group comprising, or alternatively consisting of miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b), or a precursor thereof, microencapsulated in biodegradable and biocompatible microspheres.

In other words, the invention relates to a pharmaceutical composition comprising an effective amount of at least one inhibitor of miR-92a, or a precursor thereof, microencapsulated in biodegradable and biocompatible microspheres.

"Pharmaceutical composition" means a mixture of substances suitable for administering to an individual that includes a pharmaceutical agent. For example, a pharmaceutical composition may comprise a miRNA inhibitor and a sterile aqueous solution.

In an embodiment of the composition of the invention, the said miRNA involved in angiogenesis consists of a mature miRNA.

In an embodiment of the composition of the invention, the said microRNA consists of the mature:

a) miR-92a comprising a sequence selected from the group consisting of SEQ ID No. 21, 22 or 23 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 21, 22 or 23;

b) miR-92b comprising a sequence selected from the group consisting of SEQ ID No. 24 or 25 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 24 or 25;

c) miR-17 comprising a sequence selected from the group consisting of SEQ ID No. 26 or 27 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 26 or 27;

d) miR-503 comprising a sequence selected from the group consisting of SEQ ID No. 28 or 29 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 28 or 29;

e) miR-16 comprising a sequence selected from the group consisting of SEQ ID No. 30, 31 or 32 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 30, 31 or 32;

f) miR-374 comprising a sequence selected from the group consisting of SEQ ID No. 33, 34, 35, 36, 37 or 38 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 33, 34, 35, 36, 37 or 38;

g) miR-24 comprising a sequence selected from the group consisting of SEQ ID No. 39, 40, 41 or 42 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 39, 40, 41 or 42;

h) miR-483 comprising a sequence selected from the group consisting of SEQ ID No. 43 or 44 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 43 or 44;

i) miR-34 comprising a sequence selected from the group consisting of SEQ ID No. 45, 46, 47, 48, 49 or 50 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 45, 46, 47, 48, 49 or 50;

j) miR-20 comprising a sequence selected from the group consisting of SEQ ID No. 51, 52, 53 or 54 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 51, 52, 53 or 54; and

k) miR-15 comprising a sequence selected from the group consisting of SEQ ID No. 55, 56, 57 or 58 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 55, 56, 57 or 58.

In another embodiment of the composition of the invention, the said miR-92a consists of the mature miR-92a comprising the sequence SEQ ID No. 21 or a sequence having at least 90%, preferably 95%, nucleotide identity with SEQ ID NO 21.

In another embodiment of the composition of the invention, the said miR-92a consists of the mature miR-92a comprising the sequence SEQ ID No. 22 or a sequence having at least 90%, preferably 95% nucleotide identity with SEQ ID NO 22.

In another embodiment of the composition of the invention, the said miR-92a consists of the mature miR-92a comprising the sequence SEQ ID No. 23 or a sequence having at least 90%, preferably 95%, nucleotide identity with SEQ ID NO 23.

In the sense of the present invention, the "percentage identity" between two sequences of nucleic acids means the percentage of identical nucleotides residues between the two sequences to be compared, obtained after optimal alignment, this percentage being purely statistical and the differences between the two sequences being distributed randomly along their length. The comparison of two nucleic acid sequences is traditionally carried out by comparing the sequences after having optimally aligned them, said comparison being able to be conducted by segment or by using an "alignment window". Optimal alignment of the sequences for comparison can be carried out, in addition to comparison by hand, by means of the local homology algorithm of Smith and Waterman (1981), by means of the local homology algorithm of Neddleman and Wunsch (1970, by means of the similarity search method of Pearson and Lipman (1988) or by means of computer software using these algorithms (GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI, or by the comparison software BLAST NR or BLAST P).

The percentage identity between two nucleic acid sequences is determined by comparing the two optimally-aligned sequences in which the nucleic acid sequence to compare can have additions or deletions compared to the reference sequence for optimal alignment between the two sequences. Percentage identity is calculated by determining the number of positions at which the nucleotide residue is identical between the two sequences, preferably between the two complete sequences, dividing the number of identical positions by the total number of positions in the alignment window and multiplying the result by 100 to obtain the percentage identity between the two sequences.

As intended herein, nucleotide sequences having at least 90% nucleotide identity with a reference sequence encompass those having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference sequence.

In an embodiment of the composition of the invention, the said miRNA involved in angiogenesis consists of a precursor of microRNA.

In an embodiment of the invention, the said precursor of microRNA involved in the angiogenesis consists of:

a) mir-92a-1 comprising the sequence SEQ ID No. 1, or a sequence having at least 90% nucleotide identity with SEQ ID No. 1;

b) mir-92a-2 comprising the sequence SEQ ID No. 2, or a sequence having at least 90% nucleotide identity with SEQ ID No. 2;

c) mir-92b comprising the sequence SEQ ID No. 3, or a sequence having at least 90% nucleotide identity with SEQ ID No. 3;

d) mir-17 comprising the sequence SEQ ID No. 4 or a sequence having at least 90% nucleotide identity with SEQ ID No. 4;

e) mir-503 comprising the sequence SEQ ID No. 5 or a sequence having at least 90% nucleotide identity with SEQ ID No. 5;

f) mir-16-1 comprising the sequence SEQ ID No. 6, or a sequence having at least 90% nucleotide identity with SEQ ID No. 6; and

g) mir-16-2 comprising the sequence SEQ ID No. 7, or a sequence having at least 90% nucleotide identity with SEQ ID No. 7;

h) mir-374a comprising the sequence SEQ ID No. 8, or a sequence having at least 90% nucleotide identity with SEQ ID No. 8;

i) mir-374b comprising the sequence SEQ ID No. 9, or a sequence having at least 90% nucleotide identity with SEQ ID No. 9;

j) mir-374c comprising the sequence SEQ ID No. 10, or a sequence having at least 90% nucleotide identity with SEQ ID No. 10;

k) mir-24-1 comprising the sequence SEQ ID No. 11, or a sequence having at least 90% nucleotide identity with SEQ ID No. 11;

l) mir-24-2 comprising the sequence SEQ ID No. 12, or a sequence having at least 90% nucleotide identity with SEQ ID No. 12;

m) mir-483 comprising the sequence SEQ ID No. 13, or a sequence having at least 90% nucleotide identity with SEQ ID No. 13;

n) mir-34a comprising the sequence SEQ ID No. 14, or a sequence having at least 90% nucleotide identity with SEQ ID No. 14;

o) mir-34b comprising the sequence SEQ ID No. 15, or a sequence having at least 90% nucleotide identity with SEQ ID No. 15;

p) mir-34c comprising the sequence SEQ ID No. 16, or a sequence having at least 90% nucleotide identity with SEQ ID No. 16;

q) mir-20a comprising the sequence SEQ ID No. 17, or a sequence having at least 90% nucleotide identity with SEQ ID No. 17;

r) mir-20b comprising the sequence SEQ ID No. 18, or a sequence having at least 90% nucleotide identity with SEQ ID No. 18;

s) mir-15a comprising the sequence SEQ ID No. 19, or a sequence having at least 90% nucleotide identity with SEQ ID No. 19, and

t) mir-15b comprising the sequence SEQ ID No. 20, or a sequence having at least 90% nucleotide identity with SEQ ID No. 20.

Generally speaking, an inhibitor is a molecule which represses or prevents another molecule from engaging in a reaction.

As used herein, the term "inhibitor of miR-X, or mir-X" refers to any molecule or compound that decreases or reduces the expression and/or activity of miR-X, or mir-X, or at least one precursor. This inhibition should, as a consequence, prevent neo-angiogenesis inhibition, i.e. promote neoangiogenesis so as to prevent the adverse remodelling of cardiac muscle after the infarction.

In one embodiment of the invention, the said inhibitor of a given microRNA involved in the angiogenesis is an oligonucleotide of 8-49 nucleotides in length having a sequence targeted to the said given microRNA, said microRNA being preferably selected from the group comprising miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b), or a precursor thereof.

In another embodiment, the said inhibitor of miR-92a is an oligonucleotide of 8-49 nucleotides in length having a sequence targeted to said miR-92a.

The expression "Targeted to" means having a nucleotide sequence that will allow hybridization to a target nucleic acid to induce a desired effect. In certain embodiments, a desired effect is reduction and/or inhibition of a target nucleic acid.

"Hybridize" means the annealing of complementary nucleic acids that occurs through “nucleotide complementarity”, i.e. the ability of two nucleotides to pair non-covalently via hydrogen bonding.

On some embodiments, miRNA inhibitor oligonucleotides are 8 to 49 nucleotides in length.

One having ordinary skill in the art will appreciate that this embodies oligonucleotides of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 or 49 nucleotides in length, or any range within. In some embodiments, oligonucleotides according to the invention, are 10 to 20 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies oligonucleotides of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotides in length, or any range within.

In certain embodiments, the oligonucleotide has a sequence that is complementary to a miRNA or a precursor thereof.

In one embodiment of the composition of the invention, the said oligonucleotide is an antisense oligonucleotide that is at least partially complementary to the sequence of the target miRNA involved in the angiogenesis, said target miRNA being preferentially selected from miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b), or a precursor thereof.

In another embodiment of the composition of the invention, the said oligonucleotide is an antisense oligonucleotide that is at least partially complementary to the sequence of miR-92a.

The expression "antisense oligonucleotide" refers to an oligonucleotide having a nucleotide sequence complementary to a specific nucleotide sequence (referred to as a sense sequence) and capable of hybridizing with the sense sequence.

"Complementarity" means the nucleotide pairing ability between a first nucleic acid and a second nucleic acid.

In certain embodiments, an antisense oligonucleotide has a nucleotide sequence that is complementary to a microRNA or a precursor thereof, meaning that the sequence of the antisense oligonucleotide is a least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the complement of a microRNA or precursor thereof, or that the two sequences hybridize under stringent hybridization conditions. Accordingly, in certain embodiments the nucleotide sequence of the antisense oligonucleotide may have one or more mismatched basepairs with respect to its target microRNA or precursor sequence,

and is capable of hybridizing to its target sequence. In certain embodiments, the antisense oligonucleotide has a sequence that is fully complementary to a microRNA or precursor thereof, meaning that the nucleotide sequence of the antisense oligonucleotide is 100% identical of the complement of a microRNA or a precursor thereof.

In the context of the present invention, "Complementary" means an antisense oligonucleotide having a nucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98% at least 99%, or 100%, identical to the complement of the nucleotide sequence of miR-92a, or precursor thereof, over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 or 49 nucleotides, or that the two sequences hybridize under stringent hybridization conditions.

"Percent complementarity" means the number of complementary nucleotide in a nucleic acid divided by the length of the nucleic acid. In certain embodiments, percent complementarity of an oligonucleotide means the number of nucleotides that are complementary to the target nucleic acid, divided by the length of the oligonucleotide.

In one embodiment, the antisense oligonucleotide sequence is "fully complementary" to the sequence of the target microRNA involved in the angiogenesis, preferentially selected from the group comprising miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b), and more preferentially miR-92a, or precursors thereof, which means that each nucleotide of the antisense oligonucleotide is capable of pairing with a nucleotide at each corresponding position in the target microRNA or precursor thereof.

In certain embodiment, the antisense oligonucleotide according to the invention has a sequence that is partially or fully complementary to the sequence of:

a) miR-92a comprising a sequence selected from the group consisting of SEQ ID No. 21, 22 or 23 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 21, 22 or 23;

b) miR-92b comprising a sequence selected from the group consisting SEQ ID No. 24 or 25 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 24 or 25;

c) miR-17 comprising a sequence selected from the group consisting SEQ ID No. 26 or 27 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 26 or 27;

d) miR-503 comprising a sequence selected from the group consisting SEQ ID No. 28 or 29 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 28 or 29; and

e) miR-16 comprising a sequence selected from the group consisting SEQ ID No. 30, 31 or 32 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 30, 31 or 32;

f) miR-374 comprising a sequence selected from the group consisting of SEQ ID No. 33, 34, 35, 36, 37 or 38 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 33, 34, 35, 36, 37 or 38;

g) miR-24 comprising a sequence selected from the group consisting of SEQ ID No. 39, 40, 41 or 42 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 39, 40, 41 or 42;

h) miR-483 comprising a sequence selected from the group consisting of SEQ ID No. 43 or 44 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 43 or 44;

i) miR-34 comprising a sequence selected from the group consisting of SEQ ID No. 45, 46, 47, 48, 49 or 50 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 45, 46, 47, 48, 49 or 50;

j) miR-20 comprising a sequence selected from the group consisting of SEQ ID No. 51, 52, 53 or 54 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 51, 52, 53 or 54; and

k) miR-15 comprising a sequence selected from the group consisting of SEQ ID No. 55, 56, 57 or 58 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 55, 56, 57 or 58.

In certain embodiments, the antisense oligonucleotide according to the invention has a sequence that is partially or fully complementary to the sequence of:

a) mir-92a-1 comprising the sequence SEQ ID No. 1, or a sequence having at least 90% nucleotide identity with SEQ ID No. 1;

b) mir-92a-2 comprising the sequence SEQ ID No. 2, or a sequence having at least 90% nucleotide identity with SEQ ID No. 2;

c) mir-92b comprising the sequence SEQ ID No. 3, or a sequence having at least 90% nucleotide identity with SEQ ID No. 3;

d) mir-17 comprising the sequence SEQ ID No. 4 or a sequence having at least 90% nucleotide identity with SEQ ID No. 4;

e) mir-503 comprising the sequence SEQ ID No. 5 or a sequence having at least 90% nucleotide identity with SEQ ID No. 5;

f) mir-16-1 comprising the sequence SEQ ID No. 6, or a sequence having at least 90% nucleotide identity with SEQ ID No. 6; and

g) mir-16-2 comprising the sequence SEQ ID No. 7, or a sequence having at least 90% nucleotide identity with SEQ ID No. 7;

h) mir-374a comprising the sequence SEQ ID No. 8, or a sequence having at least 90% nucleotide identity with SEQ ID No. 8;

i) mir-374b comprising the sequence SEQ ID No. 9, or a sequence having at least 90% nucleotide identity with SEQ ID No. 9;

j) mir-374c comprising the sequence SEQ ID No. 10, or a sequence having at least 90% nucleotide identity with SEQ ID No. 10;

k) mir-24-1 comprising the sequence SEQ ID No. 11, or a sequence having at least 90% nucleotide identity with SEQ ID No. 11;

l) mir-24-2 comprising the sequence SEQ ID No. 12, or a sequence having at least 90% nucleotide identity with SEQ ID No. 12;

m) mir-483 comprising the sequence SEQ ID No. 13, or a sequence having at least 90% nucleotide identity with SEQ ID No. 13;

n) mir-34a comprising the sequence SEQ ID No. 14, or a sequence having at least 90% nucleotide identity with SEQ ID No. 14;

o) mir-34b comprising the sequence SEQ ID No. 15, or a sequence having at least 90% nucleotide identity with SEQ ID No. 15;

p) mir-34c comprising the sequence SEQ ID No. 16, or a sequence having at least 90% nucleotide identity with SEQ ID No. 16;

q) mir-20a comprising the sequence SEQ ID No. 17, or a sequence having at least 90% nucleotide identity with SEQ ID No. 17;

r) mir-20b comprising the sequence SEQ ID No. 18, or a sequence having at least 90% nucleotide identity with SEQ ID No. 18;

s) mir-15a comprising the sequence SEQ ID No. 19, or a sequence having at least 90% nucleotide identity with SEQ ID No. 19, and

t) mir-15b comprising the sequence SEQ ID No. 20, or a sequence having at least 90% nucleotide identity with SEQ ID No. 20.

In certain embodiments, the antisense oligonucleotide according to the invention has a sequence that is partially complementary to the sequence of the mir-92a-1 (SEQ ID NO: 1).

In certain embodiments, the antisense oligonucleotide according to the invention has a sequence that is fully complementary to the sequence of the mir-92a-1 (SEQ ID NO: 1).

In certain embodiments, the antisense oligonucleotide according to the invention has a sequence that is partially complementary to the sequence of the mir-92a-2 (SEQ ID NO: 2).

In certain embodiments, the antisense oligonucleotide according to the invention has a sequence that is fully complementary to the sequence of the mir-92a-2 (SEQ ID NO: 2).

In one embodiment, the antisense oligonucleotide comprises a modified backbone. Examples of such backbones are provided by morpholino backbones, carbamate backbones, siloxane backbones, sulfide, sulfoxide and sulfone backbones, formacetyl and thioformacetyl backbones, methyleneformacetyl backbones, riboacetyl backbones, alkene containing backbones, sulfamate, sulfonate and sulfonamide backbones, methyleneimino and methylenehydrazino backbones, and amide backbones.

Morpholino oligonucleotides have an uncharged backbone in which the deoxyribose sugar of DNA is replaced by a six membered ring and the phosphodiester linkage is replaced by a phosphorodiamidate linkage. Morpholino oligonucleotides are resistant to enzymatic degradation and appear to function as antisense agents by arresting translation or interfering with pre-mRNA splicing rather than by activating RNase H.

A modified backbone is typically preferred to increase nuclease resistance. A modified backbone can also be preferred because of its altered affinity for the target sequence compared to an unmodified backbone. An unmodified backbone can be RNA or DNA.

Another suitable antisense oligonucleotide comprises a Peptide Nucleic Acid (PNA), having a modified polyamide backbone. PNA-based molecules are true mimics of DNA molecules in terms of base-pair recognition. The backbone of the PNA is composed of 7V-(2-aminoethyl)- glycine units linked by peptide bonds, wherein the nucleobases are linked to the backbone by methylene carbonyl bonds.

A further suitable backbone comprises a morpholino nucleotide analog or equivalent, in which the ribose or deoxyribose sugar is replaced by a 6-membered morpholino ring. A most preferred nucleotide analog or equivalent comprises a phosphorodiamidate morpholino oligomer (PMO), in which the ribose or deoxyribose sugar is replaced by a 6-membered morpholino ring, and the anionic phosphodiester linkage between adjacent morpholino rings is replaced by a non-ionic phosphorodiamidate linkage.

In yet a further embodiment, an antisense oligonucleotide of the invention comprises a substitution of one of the non-bridging oxygens in the phosphodiester linkage. This modification slightly destabilizes base- pairing but adds significant resistance to nuclease degradation.

A further suitable antisense oligonucleotide of the invention comprises one or more sugar moieties that are mono- or disubstituted at the 2', 3' and/or 5' position such as a -OH; -F; substituted or unsubstituted, linear or branched lower (CI- CIO) alkyl, alkenyl, alkynyl, alkaryl, allyl, aryl, or aralkyl, that may be interrupted by one or more heteroatoms; 0-, S-, or N-alkyl; 0-, S-, or N-alkenyl; 0-, S-or N-alkynyl; 0-, S-, or N-allyl; O-alkyl-O-alkyl, -methoxy, -aminopropoxy; -amino xy; methoxyethoxy; -dimethylaminooxyethoxy; and -dimethylaminoethoxyethoxy.

The sugar moiety can be a pyranose or derivative thereof, or a deoxypyranose or derivative thereof, preferably a ribose or a derivative thereof, or a deoxyribose or a derivative thereof. Such preferred derivatized sugar moieties comprise Locked Nucleic Acid.

An LNA is a modified RNA nucleotide wherein the ribose moiety of LNA nucleotide is modified with an extra bridge connecting 2' and 4' carbons. This enhances the base stacking and pre-organization, and significantly increases the thermal stability. This bridge "locks" the ribose in 3'-endo structural conformation, which is often found in A-form of DNA or RNA. LNA nucleotides used in the present invention can be mixed with DNA or RNA bases in the oligonucleotide whenever desired.

According to the invention, the said antisense oligonucleotide is selected in the group consisting of a ribonucleotide, a deoxyribonucleotide, a small RNA, an antagomir, a LNA, a CDNA, a PNA, a morpholino oligonucleotide or a combination thereof.

In another embodiment, the antisense oligonucleotide can consist of an antagomir.

In a preferred embodiment of the composition of the invention, the said oligonucleotide consists of an antagomir.

Antagomirs are chemically engineered oligonucleotides which are used to silence endogenous microRNA. An antagomir is a small synthetic RNA or DNA that is perfectly complementary to the specific microRNA target with either mispairing at the cleavage site or some sort of base modification to inhibit cleavage. Usually, antagomirs have some sort of modification to make it more resistant to degradation and facilitate cellular internalization. It is unclear how antagomirization (the process by which an antagomir inhibits microRNA activity) operates, but it is believed to inhibit by irreversibly binding the microRNA. Antagomirs are used to constitutively inhibit the activity of specific microRNAs.

In an embodiment of the invention, the said antagomir comprises a nucleotide sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15 or 16 contiguous nucleotides complementary to a microRNA, or a precursor thereof, the said microRNA having a sequence selected from the group consisting of SEQ ID No. 1 to 58.

In an embodiment of the invention, the said antagomir comprises a nucleotide sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15 or 16 contiguous nucleotides complementary to the mir-92a of sequence selected from the group consisting of SEQ ID No. 1, 2 or 3.

In an embodiment of the invention, the said antagomir comprises a nucleotide sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15 or 16 contiguous nucleotides complementary to the miR-92a of sequence selected from the group consisting of SEQ ID No. 21, 22 or 23.

In another embodiment, the said antagomir comprises a nucleotide sequence comprising at least 16 contiguous nucleotides complementary to the nucleotides of sequence SEQ ID No. 21.

In an embodiment of the invention, the said antagomir possesses a DNA backbone.

In said embodiment of the composition of the invention, the said antagomir comprises the sequence SEQ ID No. 59 and modifications excluding base substitutions thereof, and fragments consisting of subsequences of SEQ ID NO: 59 of at least 8 contiguous nucleotides thereof.

In another embodiment of the invention, the said antagomir possesses a RNA backbone.

In said embodiment of the composition of the invention, the said antagomir comprises the sequence SEQ ID No. 60 and modifications excluding base substitutions

thereof, and fragments consisting of subsequences of SEQ ID NO: 60 of at least 8 contiguous nucleotides thereof.

In one embodiment, the antagomir according to the invention is a fragment consisting of a subsequence of SEQ ID NO: 59 or 60 of at least 8 contiguous nucleotides thereof.

In one embodiment, the antagomir according to the invention is a fragment consisting of a subsequence of SEQ ID NO: 59 or 60 of at least 9 contiguous nucleotides thereof.

In one embodiment, the antagomir according to the invention is a fragment consisting of a subsequence of SEQ ID NO: 59 or 60 of at least 10 contiguous nucleotides thereof.

In one embodiment, the antagomir according to the invention is a fragment consisting of a subsequence of SEQ ID NO: 59 or 60 of at least 11 contiguous nucleotides thereof.

In one embodiment, the antagomir according to the invention is a fragment consisting of a subsequence of SEQ ID NO: 59 or 60 of at least 12 contiguous nucleotides thereof.

In one embodiment, the antagomir according to the invention is a fragment consisting of a subsequence of SEQ ID NO: 59 or 60 of at least 13 contiguous nucleotides thereof.

In one embodiment, the antagomir according to the invention is a fragment consisting of a subsequence of SEQ ID NO: 59 or 60 of at least 14 contiguous nucleotides thereof.

In one embodiment, the antagomir according to the invention is a fragment consisting of a subsequence of SEQ ID NO: 59 or 60 of at least 15 contiguous nucleotides thereof.

In another embodiment, the said antagomir of sequence SEQ ID No. 59 or 60 presents at least 1, 2, 3, 4, 5, 6 or 7 modified nucleotide(s) by phosphotioate bond(s) between adjacent nucleotide.

In other embodiment of the invention, the said antagomir can include 2'-O-methyl modified nucleotide, cholesterol group or any similar or equivalent modification.

Generally, if a pharmaceutical formulation containing a nucleic acid, a peptide and a protein is administered orally or parenterally, it is degraded by enzymes in the body, and the efficacy of the pharmaceutical formulation disappears quickly. Various trials have been

made to conquer the problem. One of which is to formulate a long sustained-release injectable.

In practice, research is being conducted on the use of stents as a system for the selective administration of microRNAs. However, rapid endothelialization of the stents could pose a problem with regard to the sustained release of microRNAs, especially for biological processes requiring prolonged gene modulation. In addition, liposomes and nanoparticles have been developed and used in vivo but pass to the circulatory system with risk of serious side effects have not been avoided. In addition, no studies have demonstrated efficacy and safety after percutaneous intracoronary administration without previous aortic clamp.

Another aspect of the invention is based on the use of biodegradable and biocompatible microspheres.

According to the invention, it has been designed and manufactured a release system appropriate for localised release of oligonucleotides in the cardiac region, based on the microencapsulation of said oligonucleotides with biocompatible biodegradable polymers. The invention allows to obtain microspheres substantially loaded with oligonucleotides, which have high encapsulation efficiency and no molecule modification/degradation. According to the invention, the purity and quality of the molecule, without adding either a stabilising agent or a retention substance, is preserved, thanks to the manufacturing conditions used, particularly the characteristics of the emulsion created, the concentration of the polymer solution and the relation between the volumes of the phases involved in the microencapsulation process. In addition, the microspheres have an appropriate particle-size distribution to enable them to be retained in the microvessels of the infarcted area without causing arterial emboliation and without being destroyed by macrophage phagocytic action.

An object of the present invention is to provide a sustained-release microsphere, which stably encapsulates a short chain deoxyribonucleic acid or a short chain ribonucleic acid, and is able to inhibit, for a long period, the inhibition of the expression of a specific protein, especially a protein whose inhibition is related to a disease.

Generally speaking, “biocompatible” means compatible with living cells, tissues, organs, or systems, and posing no risk of injury, toxicity, or rejection by the immune system. A biocompatible microsphere means that the microsphere, and any degradation products of the microsphere, is non-toxic to the recipient and also presents no significant

deleterious or untoward effects on the recipient's body, such as an immunological reaction at the injection site.

Generally speaking, “biodegradable” means capable of being decomposed by the action of biological agents.

A biodegradable microsphere, as defined herein, means the microsphere will degrade or erode in vivo to form smaller chemical species. Degradation can result, for example, by enzymatic, chemical and/or physical processes.

Suitable biocompatible, biodegradable polymers include, for example, poly(lactide)s, poly(glycolide)s, poly(lactide-co-glycolide)s, poly(lactic acid)s, poly(glycolic acid)s, poly(lactic acid-co-glycolic acid)s, polycaprolactone, polycarbonates, polyesteramides, polyanhydrides, poly(amino acids), polyorthoesters, polyacetyls, polycyanoacrylates, polyetheresters, poly(dioxanone)s, poly(alkylene alkylate)s, copolymers of polyethylene glycol and polyorthoester, biodegradable polyurethanes, blends and copolymers thereof.

A number of techniques to produce microparticles have been described in the prior art.

The drug release profile for a microparticle is dependent on numerous factors, including physicochemical properties of the used polymers, interactions among polymer-drug-excipients and/or morphology and composition of the resulting microparticles.

In order for the microspheres to be retained by the myocardial capillaries they must have very specific size distribution so that their destruction through phagocytosis by macrophages, on the one hand, or embolization of the arteries, on the other, is minimised; microencapsulation using a biodegradable, biocompatible polymer enables controlled release of the product from the initial hours for up to 2-3 weeks, the main period during which post-AMI ventricular remodelling occurs.

In the context of the present invention, as the intracoronary route is preferred, the average size of these microspheres makes allowance for the size of the myocardial capillaries; this varies between 5 and 20, preferentially between 5 and 15 microns, to be retained in the cardiac region, and without particles superior to 25 microns to prevent arterial embolization.

In one embodiment of the invention, the said microspheres are presenting a diameter which does not exceed 25 µm.

In one embodiment, 50 to 100% of the microspheres are comprised in the range of 5 to 25 µm and no particles upper than 25 µm

In a preferred embodiment, 60 to 100% of the microspheres are comprised in the range of 5 to 25 μm and no particles upper than 25 μm.

In another preferred embodiment, 70 to 100% of the microspheres are comprised in the range of 5 to 25 μm and no particles upper than 25 μm.

In a more preferred embodiment, 80 to 100% of the microspheres are comprised in the range of 5 to 25 μm and no particles upper than 25 μm.

According to the invention, the average diameter of the microspheres is in the range of 5 to 20 μm.

According to the invention, the average diameter of the microspheres is in the range of 5 to 15 μm.

In an alternative embodiment, the said microspheres are presenting an average diameter from 8 to 11 μm.

The microspheres according to the invention load a high amount of the said inhibitor of microRNA in a biodegradable and biocompatible polymer to sustained release the drug in the infarcted area.

The inhibitor of microRNA loading is from about 1 to 20% (w/w), preferably from 1 to 15% (w/w), and more preferably 1 to 10 and still more preferably from 5 to 10%. The drug integrity is conserved.

In one embodiment, the microspheres according to the invention are incorporating from 1% to 15 % w/w of inhibitor.

In another embodiment, the microspheres according to the invention are incorporating from 5% to 15 % w/w of inhibitor.

Still in another embodiment, the microspheres according to the invention are incorporating from 1% to 10%, preferably from 5% to 10 % w/w of inhibitor.

Another characteristic of the invention relates on the nature of the polymer used for the generation of the microspheres.

In an embodiment of the invention, the said microspheres are made of a polymer consisting of poly-d,l-lactide (PLA). In an embodiment, the said microspheres are made of PLA as the sole polymer. In an embodiment, the said microspheres are made of PLA which is blended with one or more other biocompatible polymers.

In another embodiment of the invention, the said microspheres are made of a copolymer consisting of poly-d,l-lactide-co-glycolide (PLGA). In an embodiment, the said microspheres are made of PLGA as the sole polymer. In an embodiment, the said

microspheres are made of PLGA which is blended with one or more other biocompatible polymers.

Still in another embodiment of the invention, the said microspheres are made of a blend of polymers consisting of poly-d,l-lactide-co-glycolide (PLGA) and poly-d,l-lactide (PLA).

By the expression "blend", it must be understood that a mixture of two or more polymers is realized before the dissolution of the said mixture into the same organic solvent.

The man skilled in the art will easily understand that, contrary to the PLGA copolymer, the ratio of lactide:glycolide in the PLA polymer is comprised between 100:0 molar ratio.

Regarding the PLGA copolymer, the ratio of lactide:glycolide in the PLGA polymer is comprised between 50:50 to 95:5 molar ratio.

In a preferred embodiment, the ratio of lactide:glycolide in the PLGA copolymer is comprised between 50:50 to 90:10 molar ratio, and preferentially between 50:50 to 80:20 molar ratio.

In an embodiment of the invention, the inherent viscosity of the polymer is comprised between 0.1 and 0.7 dl/g.

In a preferred embodiment, the inherent viscosity of the polymer is comprised between 0.15 and 0.7 dl/g, preferentially from 0.15 to 0.5 dl/g.

This aspect is of interest in the sense that inherent viscosity is related with the polymer molecular weight and, therefore, influences in the inhibitor release rate from the polymeric microspheres.

Accordingly, it is also an object of the present invention to provide a method for producing a microencapsulated inhibitor of miR-92a which does not include any adjuvant and which consists of a single population of particles in terms of polymeric composition.

Thus, another aspect of the invention is a method for microencapsulating an inhibitor of microRNA in polymeric microspheres, comprising: (a) dissolving the inhibitor of microRNA in purified water, without any stabilizer (b) dissolving polymer in an organic solvent; (c) adding (a) to (b) to produce a first emulsion; (d) adding the emulsion of step (c) to an aqueous solution containing a surfactant and an osmotic agent to produce a second emulsion; (e) hardening and harvesting the resulting microspheres of step (d); and (f) drying.

More particularly, the invention also relates to a method for producing a composition as above described, characterized in that it comprises the following steps:

a) dissolving the inhibitor of miRNA in purified water, without any stabilizer;

b) dissolving polymer in an organic solvent;

c) adding (a) to (b) to produce a first emulsion;

d) adding the emulsion of step (c) to an aqueous solution containing a surfactant and an osmotic agent to produce a second emulsion; and

e) hardening and harvesting the resulting microspheres of step (d); and

f) drying the obtained microspheres.

Another aspect of the invention consists of the use of the composition according to the invention in the treatment of the myocardial infarction.

In other word, the invention relates to a composition comprising an effective amount of at least one inhibitor of microRNA, or a precursor thereof, wherein said inhibitor is microencapsulated into polymeric biodegradable and biocompatible microspheres for use in the treatment of myocardial infarction.

In a preferred embodiment, the said myocardial infarction consists of the acute myocardial infarction.

Still another aspect of the invention relates to a method of reversing or preventing ventricular remodelling in a subject in need thereof comprising administering to said subject an effective amount of a composition as above described.

In other word, the invention relates to a composition comprising an effective amount of at least one inhibitor of microRNA, or a precursor thereof, wherein said inhibitor is microencapsulated into polymeric biodegradable and biocompatible microspheres for use in a method of reversing or preventing ventricular remodelling in a subject in need thereof.

As already mentioned, the composition according to the invention is suitable for an administration by intracoronary route.

For administration by intracoronary route, the microspheres must be suspended in an appropriate vehicle, either a saline solution (PBS) with or without a surfactant or in another appropriate vehicle for intravenous administration. Suitable dispersants include, for example, surfactants such as polysorbate 80, polysorbate 20, polyoxyethylene hydrogenated castor oil 60, carboxymethylcellulose, or polysaccharides such as sodium alginate; it is also possible add an isotonizing agent such as sodium chloride, mannitol, sorbitol or glucose, for example. Given the size of coronary arteries, the concentration of

microspheres in the administration medium is adjusted in order to limit blood flow alteration and prevent the risk of arterial embolization. This concentration can vary between 0.05% and 1%, preferably between 0.1% and 0.5%. Administration can be by a single injection or repeated injections, followed optionally by a saline injection. The administration could be carried out after the percutaneous coronary angioplasty, without limitation, using the same catheter.

The method of the invention is characterized in that said administration consists of an administration by intracoronary route.

This aspect is of particular interest as it addresses several presupposed limitations of direct intravenous administration of compounds such as oligonucleotide like antagomir. Among other presupposed limitation, it can be mentioned i) low-level biosafety due to the ubiquity and low organ specificity of miRNAs, ii) high doses and repeated injections for the microRNA inhibitor to produce its effect, and iii) high theoretical cost of the calculated intravenous dose.

Surprisingly, as it will be apparent after the reading of the following examples, all these issues are addressed by the invention.

More particularly, it is demonstrated that:

a) it is possible to selectively administer the encapsulated antagomir in the artery supplying the diseased tissue (see Example 5);

b) microspheres are retained in the coronary. (see Example 6);

c) intra-arterial administration of microspheres is used for tumour embolization, enabling permanent blood flow interruption and preventing tumour progression, optionally in combination with active release substances. Taking into consideration these elements, the existence of the risk of embolization was considered. For this reason, studies were designed with the objective of ascertaining that the microspheres cause no damage to cardiac muscle or produce any significant alterations in coronary flow rate. (see Example 7);

d) good stability of the inhibitor of microRNA in the vehicle and sustained release of said inhibitor from microspheres; this is demonstrated by its biological effect while inhibiting microRNA for up to 10 days after it is administered (Example 8);

e) administration of microspheres with the miRNA inhibitor promotes contractile recovery of damaged tissue and prevents the occurrence of adverse post-infarction remodelling. (see Example 9);

f) with localised administration of microspheres, the inhibitor dose could be reduced to a single injection, which would presume a significant reduction of potential side effects and a clear reduction in cost.

The availability of an appropriate vehicle/system for the controlled administration, delivery and release of microRNA inhibitors according to the invention has the following advantages:

- Enhanced biosafety, since bio-distribution of the drug by tissue and organs that are not the treatment target, is limited

- Avoids repeated intravenous injections i) reduces hospital admissions and outpatient hospital visits, by enhancing the quality of patient support, ii) avoids the need for prolonged mainlining for drug administration as well as potential risks resulting from this and iii) minimizes the risks inherent in intravenous administration of products (infections, localised reactions ...)

- Dose reduction allows the reduction in related dose-dependent adverse effects

- Reduction of costs due to a reduction in the doses required as well as the staff and equipment required for repeated injections

In another embodiment, the invention relates to a population of biodegradable and biocompatible microspheres for use in the treatment or prevention of ventricular remodelling after myocardial infarction, wherein said microspheres:

- have an average diameter comprised between 5 and 15 μm;
- are made of poly-d,l-lactide-co-glycolide (PLGA) ; poly-d,l-lactide (PLA) or a blend thereof;
- are incorporating from 1% to 10 % w/w of a therapeutic agent capable of preventing ventricular remodelling

wherein said therapeutic agent consists of an inhibitor of a microRNA involved in angiogenesis, preferentially a microRNA selected from the group consisting of miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b) and more preferentially miR-92a, or a precursor thereof, wherein said inhibitor of microRNA is preferentially an antagomir.

The invention also relates to a kit comprising at least i) a composition and/or microspheres according to the invention and ii) a syringe or vial or ampoule in which the composition is disposed.

In an embodiment, the kit of the invention further comprises a solvent disposed in a solvent container. The solvent container may be a vial, an ampoule or a prefilled syringe

The microspheres and the solvent may be disposed in a double compartment prefilled syringe.

In an embodiment, the kit of the invention may comprise the microspheres in a vial and the solvent in a separate vial.

In an embodiment, the kit of the invention may comprise the microspheres in a vial and the solvent in a separate ampoule.

In an embodiment, the kit of the invention may comprise the microspheres in a vial and the solvent in a prefilled syringe.

In an embodiment, the kit of the invention may comprise the microspheres in a prefilled syringe and the solvent in a separate vial.

In an embodiment, the kit of the invention may comprise the microspheres in a prefilled syringe and the solvent in a separate ampoule.

In an embodiment, the kit of the invention may comprise the microspheres and the solvent separately in a double compartment syringe.

The invention will be better understood in respect to the following examples.

Example 1: Preparation of Microspheres loaded with antagomir-92a

Microspheres were prepared by w/o/w emulsion/solvent evaporation method using a 50:50 PLGA copolymer, intrinsic viscosity of about 0.2 dL/g, which contains free carboxyl end groups. 3 ml of methylene chloride were added to 0.6 g or PLGA. 0.3 ml of a concentrated solution of Antagomir-92a (I-Ssc-miR-92a; molecular mass: 5366 g/mol (also referred as Da); sequence: CCGGGACAAGTGCAAT; DNA Bases: 9; LNA Bases: 7; fabricant: IDT (Exiqon)) (222 mg/ml) in purified water was added to the PLGA organic solution and emulsified by sonication for 20 s. This primary emulsion was added to an external phase consisting of an aqueous solution of 1% (w/v) polyvinyl alcohol and 1% (w/v) of sodium chloride and homogenised for 60 s at about 10300 rpm. The second emulsion (w/o/w) obtained was added to a volume of purified water and the methylene chloride was allowed to evaporate by stirring. The obtained microspheres were collected

by centrifugation, washed twice with purified water, and then freeze dried. Average diameter of microspheres was 9 μm, (82% between 5-25 μm and 0% upper 25 μm) while encapsulation efficiency was 74%.

A picture of the obtained microspheres is represented in Figure 1.

Figure 2 illustrates the distribution of the microsphere size.

Example 2: Preparation of Microspheres loaded with RNA

Microspheres were prepared by w/o/w emulsion/solvent evaporation method using a 50:50 PLGA copolymer, intrinsic viscosity of about 0.2 dL/g, which contains free carboxyl end groups. 3 ml of methylene chloride were added to 0.6 g or PLGA. 0.3 ml of a concentrated solution of RNA (222 mg/ml) (RNA Sigma 5000-10000Da) in purified water RNAsa free was added to the PLGA organic solution and emulsified by sonication for 20 s. This primary emulsion was added to an external phase consisting of an aqueous solution RNAsa free of 1% (w/v) polyvinyl alcohol and 5% (w/v) of mannitol and homogenised for 60 s at about 10300 rpm. The second emulsion (w/o/w) obtained was added to a volume of purified water RNAsa free and the methylene chloride was allowed to evaporate by stirring. The obtained microspheres were collected by centrifugation, washed twice with purified water RNAsa free, and then freeze dried. Average diameter of microspheres was 10 μm, (86% between 5-25 μm and 0% upper 25 μm) while encapsulation efficiency was 73%.

Example 3: Preparation of placebo Microspheres

Microspheres were prepared by w/o/w emulsion/solvent evaporation method using a 50:50 PLGA copolymer, intrinsic viscosity of about 0.2 dL/g, which contains free carboxyl end groups. 3 ml of methylene chloride were added to 0.6 g or PLGA. 0.3 ml of purified water was added to the PLGA organic solution and emulsified by sonication for 20 s. This primary emulsion was added to an external phase consisting of an aqueous solution of 1% (w/v) polyvinyl alcohol and 1% (w/v) of sodium chloride and homogenised for 60 s at about 10300 rpm. The second emulsion (w/o/w) obtained was added to a volume of purified water and the methylene chloride was allowed to evaporate by stirring. The obtained microspheres were collected by centrifugation, washed twice with purified water, and then freeze dried. Average diameter of microspheres was 7 μm, (84% between 5-25 μm and 0% upper 25 μm).

Example 4: Preparation of Microspheres loaded with albumin fluorescein isothiocyanate

Microspheres were prepared by w/o/w emulsion/solvent evaporation method using a 50:50 PLGA copolymer, intrinsic viscosity of about 0.2 dL/g, which contains free carboxyl end groups. 1 ml of methylene chloride was added to 0.2 g or PLGA. 0.1 ml of an albumin fluorescein isothiocyanate aqueous solution (20 mg/ml) was added to the PLGA organic solution and emulsified by sonication for 15 s. This primary emulsion was added to an external phase consisting of an aqueous solution of 1% (w/v) polyvinyl alcohol and 1% (w/v) and homogenised for 60 s at about 10300 rpm. The second emulsion (w/o/w) obtained was added to a volume of purified water and the methylene chloride was allowed to evaporate by stirring. The obtained microspheres were collected by centrifugation, washed twice with purified water, and then freeze dried. Average diameter of microspheres was 9 μm, (91% between 5-25 μm and 0% upper 25 μm).

Example 5: Study of selective administration in the artery supplying the target tissue

After triggering an AMI in a Large White pig, 30 mg of microspheres containing fluorescent albumin, prepared as indicated in example 4, were administered by intra-coronary route, by means of a 2.5/12 coaxial balloon positioned in the artery responsible for the AMI, which supplies the infarcted area. The microspheres were suspended *in situ* in 10 ml of normal saline solution containing Tween-80; administration was performed in 2 consecutive injections of 5 ml, each followed by 5 ml of normal saline solution. Experiments showed that the encapsulated antagomir can be selectively administered in the artery supplying the diseased tissue.

Example 6: Study of retention of the microspheres in the capillaries of the diseased tissue without allowing outflow into the bloodstream

On a pig model, 4 experiments were conducted by administering by intra-coronary route through a coaxial balloon positioned in the medial anterior descending branch, 2 injections with 5 ml each of fluorescent microspheres prepared according to example 4. The 4 animals were euthanized and myocardial samples of the tissue adjoining the anterior descending branch and the control tissue irrigated by other coronary arteries were obtained. The samples were observed through an optical fluorescence microscope and the presence

of microspheres retained in the capillaries of the damaged cardiac muscle as well as their absence in the control tissue was demonstrated.

To rule out systemic biodistribution, in two of the previous animals, besides ischemic and control myocardial tissue, 5 replicate samples from lung, spleen and liver were obtained and visualized by optical microscopy with light B. Fluorescence was exclusively detected in anterior myocardial wall. This analysis revealed that microspheres are retained in the heart avoiding systemic release of antagomir92a (reduction of side effects).

Example 7: Study of retention of microspheres in the capillaries of diseased tissue without damaging the target tissue itself

Experiments were conducted to investigate the potential local heart toxicity and the therapeutic safety range of dose. To detect local ischemic damage to the cardiac muscle 2 paired piezoelectric crystals, which are highly sensitive in their ability to detect ischemia were employed. When cardiac muscle tissue is affected by ischemia, the remaining tissue becomes dyskinetic and swells; this, along with the blood pressure produced by the remaining contiguous healthy tissue causes the microcrystals to separate and move further away from each other. In two pigs after performing a thoracotomy and a pericardiectomy two pairs of microcrystals were inserted, one control pair in the lateral region and one pair in the anterior region supplied by the anterior descending branch, through which the microspheres were administrated. For each pair of medium crystals the distance between them at two points during the cardiac cycle was measured: at end-diastole (**EDL**) and end-systole (**ESL**). The relation between EDL and ESL is expressed by the parameter **SS** (systolic shortening: (EDL – ESL) / EDL. When the left ventricular contraction is completely dissipated EDL = ESL and SS = 0. The normal values range between 0.2 ± 0.1. As shown in the attached illustration, minimal and transient oscillations after each injection lasting a few seconds were induced with the dose of the study, corresponding to the first and second injections. Furthermore and surprisingly, no local side effects were observed with repeated intracoronary injections of fluorescent microspheres prepared according to example 4 reaching 14 times the dose of the study. No limiting maximum dose was associated with irreversible ischemic damage, hemodynamic repercussion or arrhythmias.

In addition, to detect changes in coronary flow a flow sensor was positioned in middle LAD measuring coronary flow. No significant changes in coronary flow were observed after intracoronary injections.

Results are illustrated by Figure 3 wherein 120mg of microspheres were injected and by Figure 4 wherein 240 mg of micropsheres were injected.

Example 8: Study of the molecular effect of a single intra-coronary injection of microspheres with a small antagomir dose

In order to demonstrate that small doses of microencapsulated antagomir could produce a molecular response, the miR-92a expression in vivo, in ischemic and control tissue, was measured after intracoronary encapsulated antagomir-92a administration. In 3 pigs, 60 mg of microspheres containing antagomir-92a prepared according to example 1 (0.1 mg/Kg) were delivered in LAD. Animals were euthanized at one, three and 10 days after treatment and expression of miR-92a and endogenous microRNA as controls (miR-123, 203, and 126) were quantified in 2 replicate infarcted and control samples by total RNA isolation and real-time quantitative RT-PCR using specific primers (see Figure 5).

In infarcted tissue, miR-92a expression resulted down-regulated by 8-fold in comparison with control tissue whereas expression of endogenous miRs was not affected by the treatment. The inhibition began to be present as early as 1 day and it was still present at day 10, with 5 times lower expression levels than in the control area.

No significant regulation was detected in endogenous miRs. These results reveals that the vehicle/system provides adequate conditions for enabling controlled delivery and release of antagomir-92a, thereby producing a sustained inhibition of microRNA-92a with a single intracoronary administration.

These results, represented in Figure 6, also confirm that the antagomir is not degraded during the microsphere manufacturing process.

Example 9: Study of the biological effect of a single intra-coronary injection of microspheres containing low doses of antagomir

In order to demonstrate whether the molecular effect of microsphere-transported antagomir-92a is accompanied by a biological effect, a pre-clinical study was conducted with 26 adult minipigs. The purpose of this study was to investigate whether inhibition of mir-92a by selective intracoronary encapsulated antagomir-92a administration leads to enhance of angiogenesis in infarcted area, and thus preventing the occurrence of ventricular remodelling.

3 formulations were administered:

- Saline solution (control formulation)

- Placebo microspheres prepared according to example 3

- Antagomir-92a microspheres prepared according to example 1, at one antagomir dose of 3 mg /minipig.

4 weeks after treatment, significant higher vascular density in the necrotic area was detected in those animals receiving encapsulated antagomir-92a compared to controls, thereby confirming the proangiogenic activity of antagomir-92a observed in previous study (161.57±58.71 vs 68.49±23.56 in placebo group vs 73.91±24.97 in saline group, p=0.001) ii) the vascular density (see Figure 7).

Microvascularity increased within both, the infarct zone and the peri-infarct rim. Lower microvascular resistance index in those treated animals was consistently demonstrated (200.67±104.46 vs 511.73±202.1 in controls, p=0.007) and it was significantly correlated with vascular density (R^2 0.41, p=0.02). (see Figure 8).

The baseline microcirculatory resistance (baseline MR) and the true microcirculatory resistance (TMR (hyp)) were significantly lower in the treated group compared with controls (7.47±1.33 vs 19.62±2.98, p=0.005 and 5.0±1.15 vs 14.49±2.4, p= 0.006 respectively). Baseline and true microcirculatory resistance were significantly correlated with vascular density (R2 0.35, p=0.033 and R2 0.31, p=0.047 respectively (see Figure 9).

These data indicate that encapsulated antagomir-92a induces sustained angiogenesis in vivo.

Having found growth vessels, its potential benefits in healing process that occurs after an AMI was therefore further investigated. To determine the effects of encapsulated antagomir-92a on ventricular remodelling, morphological and structural parameters by ex vivo magnetic resonance imaging (CMR) and functional parameters analysed by intravascular echocardiography (IVE), in the treated and non-treated groups were compared. Significant more percentage of animals with anterior and septoapical dyskinesia was present in IVE in controls (p=0.03) (see notably Figure 10) with also significant higher thinning of the injured ventricular wall and adverse remodelling morphometry changes in left ventricle in ex-vivo CMR in comparison with treated animals (Table 3).

More particularly, Figure 10 illustrates the results of the analysis of regional wall motion dysfunction by intravascular echocardiography (IVE). IVE was performed by using a Vivid Q ultrasound imaging machine (GE Healthcare, Belford, UK) and an AcuNav 10F ultrasound catheter (Siemens) placed in the apex of the right ventricle.

The results of the study show that the administration of antagomir-92a microspheres is associated with a statistically significant reduction in adverse remodelling following acute myocardial infarction.

Table 3: Parameters of left ventricular remodelling in CMR

	Saline (N=6)	Placebo ME (N=5)	Antagomir-92a ME (N=6)	P
Number of infarctedCMR slices	4.8±0.3	4.8±0.4	5.3±0.2	0.38
$T_{maxinfarctedwall}$, mm	6.07±0.9	5.61±0.5	9.01±0.6	0.006
$T_{normal\ posterior\ wall}$, mm	13.23±0.5	13.52±1.8	11.82±0.7	0.49
Percentage of minimumthinning, %	54.79±4.9	56.74±4.1	22.71±5.5	0.000
$T_{mininfarctedwall}$, mm	3.17±0.4	4.02±0.9	4.35±0.5	0.33
Percentage of maximumthinning, %	76.40±2.18	69.86±4.72	62.54±4.19	0.05
Length of the thinning wall, mm	32.2±1.8	31.7±4	20.5±3.6	0.03
D_R/D_N	1.93±0.2	2.02±0.2	1.29±0.1	0.03
D_N,mm	14.88±0.68	13.78±1.59	17.5±1.37	0.12
Adverse remodeling% (n)	83.3 (5)	80 (4)	16.7 (1)	0.03

Encapsulated antagomir-92a prevents adverse left ventricular remodelling 1 month after acute myocardial infarction. The different remodelling parameters calculated in all infarcted slices of ex-vivo CMR in each minipig were dtermined. Representative L2 slice (being L1 the apex) of four minipigs are shown. $T_{max\ infarcted\ wall}$ = mean maximum infarcted wall thickness calculated as $\sum$ of the maximum infarct wall thickness in each slice divided by the number of affected slices; $T_{normal\ posterior\ wall}$ = mean thickness of normal posterior wall measured just beside the insertion of posterior papillary muscle calculated as $\sum$ of the wall posterior thickness in each affected slice divided by de number of affected slices; mean percentage of minimum thinning calculated as $[100 - (T_{max\ infarcted\ wall}/T_{normal\ posterior\ Wall} \times 100)]$; $T_{min\ infarcted\ wall}$ = mean minimum infarcted wall thickness calculated as $\sum$ of the minimum infarct wall thickness in each affected slice divided by the number of affected slices; mean percentage of maximum thinning calculated as $[100 - (T_{min\ infarcted\ wall}/T_{normal\ posterior\ wall} \times 100)]$; D_R: mean maximal diameter between infarcted wall and contralateral normal wall calculated as $\sum$ of the maximal diameter between infarcted wall in each infarcted slice divided by the number of affected slices; D_N: mean maximal diameter between normal walls, forming a right angle with D_R and drawn nearest thecenterofthe ventricularcavity, calculated as $\sum$ of the maximal diameter between normal walls in each infarcted slice divided by the number of affected slices; D_R/D_N: mean sphericity index calculated as $\sum$ of the D_R/D_N of each infarcted slice divided by the number of affected slices. Data of the table are expressed as the mean ± s.e.m.

The results of a epresentative CMR show that :

***A**: Cardiac NMR and IVE of minipig 14 (death immediately after induction of AMI)*: Owing to the occurrence of death immediately following the AMI, there was not enough time for the remodelling process to be triggered. This is why a concentric left ventricle was seen to have similar dimensions in all of the segments.

***B**: Cardiac NMR and IVE of the minipig 20 to 30 days post-AMI: evidence of adverse ventricular remodelling:* One month after the AMI, extreme emaciation of the anterior and septal sections was observed on the CMR as well as an aneurysm formation with dyskinesia on the IVE which is typical of post-AMI adverse remodelling.

***C**: Cardiac NMR and IVE of the minipig 22 to 30 days post-AMI: no ventricular remodelling:* One month after AMI, slight reduction of the parietal region in the anterior and septal areas was observed without aneurysm formation and without dyskinesia in the IVE. This is a typical case of favourable repair reactions after AMI.

Example 10: Study of the induction of vascular tumours or effects in short-term mortality of encapsulated antagomir-92a

No vascular tumors were observed in necropsy analysis performed to all animals, thereby suggesting the absence of ectopic systemic suppression of microRNA-92a in other organs at distance. The mortality of the study was of 23 %. No differences were observed in short-term mortality. Only one minipig allocated to encapsulated antagomir92a died (p=0.39).

Table 4

N= 26	Saline (n=9)	Placebo ME (n=9)	Antagomir-92a ME (n=8)
1 month follow-up	6	7	7
Death	3	2	1

Example 11: Study of the proarrhythmic profile of encapsulated antagomir-92a.

In order to know the arrhythmogenic potential of encapsulated antagomir-92a, all arrhythmic events during the procedures were recorder and analyzed by Collect 5S software (GE). Moreover, to address this issue, an insertable loop recorder was randomly implanted in 10 of the 26 minipigs of the study to detect potential episodes of arrhythmia until sacrifice, at one month postinfarction and treatment. No higher number of maligne tachyarrhythmias nor bradiarrhythmias were observed in treated group in comparison with controls, indicating that intracoronary encapsulated antagomir-92a doesn´t exert a proarrhythmic effect.

Table 5: Arrhythmias detected during the study

	Saline (n=9)	Placebo ME (n=9)	Antagomir-92a ME (n=8)	p
Ischemic phase (n= 26)				
No arrhythmias, n(%) Arrhythmias, n(%) PVC, n NSVT, n Ventricular fibrilation, n	2 (22.2) 7 (77.8) 5 0 3	0 (100) 7 1 3	1 (12.5) 7 (87.5) 5 1 3	0.37
Reperfusion phase (n=26)				
No arrhythmias, n(%) Arrhythmias, n(%) Sinusal pauses, n Nodal rhythm,n IVR, n PVC, n NSVT, n	5 (55.6) 4 (44.4) 1 1 0 4 0	3 (33.3) 6 (66.7) 1 0 2 3 0	3 (37.5) 5 (62.5) 0 0 1 3 1	0.6
During 30 days after AMI (n=10)				
Implantable loop recorder	n=4	n=3	n=3	0.88
No arrhythmias, n(%) Arrhythmias, n(%) Sinusal taquicadia Sinusal pausa PVC or PSVC Not assessable	0 (0) 2 (50) 2 0 0 2 (50)	0 (0) 3 (100) 3 1 0 0	0 (0) 3 (100) 3 0 1 0	0.38

PVC: premature ventricular complexes, NSVT: non-sustained ventricular taquicardia,

IVR: idioventricular rhythm,

PSVC: premature supraventricular complexes

AMI: acute myocardial infarction

Example 12: evaluation of the effects of encapsulated antagomir 92a and non encapsulated antagomir-92a on the expression of miR92a *in vitro*.

12.1: Material and Method

a. Cells

The human umbilical vein cell line, EA.hy926, established by fusing primary human umbilical vein cells with a thioguanine-resistant clone of lung cells A549 (ATCC® CRL-2922™) has been used.

b. Treatment

Approximately 500 000 EA.hy926 cells were seeded onto six well plates and incubated under standard condition (37°C, 5% CO2) in RPMI 1640 supplemented in 10% fetal bovine serum (FBS) and 2 mM L-glutamine (Sigma, L'Isle d'abeau, France) Medium was then replaced by fresh completed RPMI medium containing the respective antagomirs and their respective controls (PBS or microspheres). EA.hy926 cells have been treated with either, antagomir 92a (free and three batches of Antagomir92a microspheres), encapsulated antagomir 17 or encapsulated antagomir 20 at 10 and 150 nM. Cells were incubated for a further 24 h before harvesting for RNA extraction. Total RNA was extracted and the expression of miRNAs was quantified by means of quantitative RT-PCR.

c. RNA Extraction

miRNAs were isolated from EA.hy926 cells and mini-pig tissues using Qiagen RNeasy mini-preps (ref. 74106) and RNeasy+ universal kits (ref. 73404), respectively, according to manufacturers instructions (Qiagen, Courtaboeuf, France). The quantity and purity of extracted RNA were assessed using a NanoDrop ND 1000 spectrophotometer (Labtech International, Paris, France).

d. Reverse transcription of miRNAs

miRNAs were reverse-transcribed, using the TaqMan MicroRNA Reverse Transcription Kit (Life Technologies, ref. 4366596), in a final volume of 15 µl containing 5 ng of total RNA and the specific miRNA probe. The samples were incubated at 16°C for 30 min and 42°C for 30 min, and reverse transcriptase was inactivated by heating at 85°C for 5 min and cooling at 4°C forever.

e. Real-Time RT-PCR

Theoretical Basis. Quantitative values are obtained from the Ct number at which the increase in signal associated with exponential growth of PCR products starts to be detected (using the QuantStudio 6 and 7 Flex Software, according to the manufacturer's

manual). To control the differences in amounts of starting material, the data were normalized to the geometric mean of 2 endogenous controls (miRNA103 and miRNA191) which expression levels have been empirically shown not to change as a function of treatment. Value of the target miRNA was subsequently normalized such that the value of the target miRNA in the control equals a value of 1. Results were expressed using the ΔΔCt calculation method (RQ analysis software, Applied Biosystems®).

PCR Amplification. All PCR reactions were performed using a QuantStudio™ 6 Flex Real-Time PCR System and TaqMan probes (Applied Biosystems®). The thermal cycling conditions comprised an initial denaturation step at 95°C for 10 min and 45 cycles at 95°C for 15 s and 65°C for 1 min. Samples were tested in duplicate.

12.1: Results

miR-92a expression is inhibited by both antagomir-92a free and encapsulated at 10 nM with an approximate 90% reduction.

miR-92a expression is undetectable after both antagomir-92a free and encapsulated at 150 nM.

Neither miR-17 or miR-20a expression was significantly reduced by antagomir-92a treatment.

These data are summarized in Figure 11.

Example 13: evaluation *in vitro* of the effects of three encapsulated antagomirs (antagomir 17, 20a and 92a) on their respective expression of miRs.

13.1 Preparation of Microspheres loaded with antagomir-17

Microspheres were prepared by an emulsion/solvent evaporation method using a 50:50 PLGA copolymer (i.v. 0.2 dL/g). A solution of antagomir-17 (HSA-miR-17-5p; molecular mass: 5305Da; sequence: CTGCACTGTAAGCACT; from Exiqon) was emulsified in the PLGA organic solution. The obtained emulsion was in turn incorporated in a dispersing aqueous phase and homogenised to obtain the desired particle size. Finally, after the solvent evaporation, the obtained microspheres were freeze-dried. The average diameter of microspheres was 10 μm and the antagomir-17 content was 7.3%.

13.2 Preparation of Microspheres loaded with antagomir-20a

Microspheres were prepared by an emulsion/solvent evaporation method using a 50:50 PLGA copolymer (i.v. 0.2 dL/g). A solution of antagomir-20a (HSA-miR-20a; molecular mass: 5289Da; sequence: CTGCACTATAAGCACT; from Exiqon) was emulsified in the PLGA organic solution. The obtained emulsion was in turn incorporated in a dispersing aqueous phase and homogenised to obtain the desired particle size. Finally, after the solvent evaporation, the obtained microspheres were freeze-dried. The average diameter of microspheres was 10 µm and the antagomir-20a content was 6.8 %.

13.3 Results

Material and method is the salme as in Example 12.

- miR-17 is inhibited at 76% by encapsulated antagomir-17 at 10 nM. miR17 expression is totally abolished by encapsulated antagomir-17 treatment at 150 nM.

- miR-20a is inhibited at 7% by encapsulated antagomir-20a treatment at 10 nM and at 87% by encapsulated antagomir-20a treatment at 150 nM.

Results are represented in Figure 12.

Example 14: evaluation *in vitro* of three batches of Antagomir92a microspheres, with the characteristics of load, size and ratio Lactide/Glycolide.

14.1 Preparation of Microspheres with low antagomir-92a loading (L13250 : Polymer : RESOMER RG502H)

Microspheres were prepared by an emulsion/solvent evaporation method using a 50:50 PLGA copolymer (i.v. 0.2 dL/g) as described in example 1 but using lower initial amount of drug and a higher agitation speed in order to prepare smaller microspheres with low antagomir-92a content. The average diameter of microspheres was 7 µm and the antagomir-92a content was 1.5 %.

14.2 Preparation of Microspheres with high antagomir-92a loading (L13262 : Polymer : RESOMER RG502H)

Microspheres were prepared by an emulsion/solvent evaporation method using a 50:50 PLGA copolymer (i.v. 0.2 dL/g) as described in example 1 but using higher initial amount of drug and a lower agitation speed in order to prepare bigger microspheres with

high antagomir-92a content. The average diameter of microspheres was 15.6 μm and the antagomir-92a content was 9.8 %.

14.3 Preparation of Microspheres loaded with antagomir-92a using a long-lasting polymer (L13230: Polymer : LACTEL B6006)

Microspheres were prepared by an emulsion/solvent evaporation method as described in example 1 but using a 85:15 PLGA copolymer with a high molecular weight (i.v. 0.64 dL/g), intended for a slow drug release. The average diameter of microspheres was 12 μm and the antagomir-92a content was 3.1%.

14.4 Results

The three batches of antagomir-92a microspheres, with the characteristics of load, size and ratio Lactide/Glycolide have been tested.

According to microsphere content L13250 has been tested at 2 and 30 nM, L13262 at 14 and 210 nM, and L13230 at 4 and 66 nM,

L13250, L13262 and L13230 totally abolished miR92a expression.

CLAIMS

1. Composition comprising an effective amount of at least one inhibitor of a miRNA involved in the angiogenesis, or a precursor thereof, wherein said inhibitor is microencapsulated into polymeric biodegradable and biocompatible microspheres.

2. The composition of claim 1, wherein said miRNA is selected in the family comprising miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b).

3. The composition of claim 1, wherein said miRNA consists of the mature:

a) miR-92a comprising a sequence selected from the group consisting of SEQ ID No. 21, 22 or 23 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 21, 22 or 23;

b) miR-92b comprising a sequence selected from the group consisting of SEQ ID No. 24 or 25 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 24 or 25;

c) miR-17 comprising a sequence selected from the group consisting of SEQ ID No. 26 or 27 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 26 or 27;

d) miR-503 comprising a sequence selected from the group consisting of SEQ ID No. 28 or 29 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 28 or 29;

e) miR-16 comprising a sequence selected from the group consisting of SEQ ID No. 30, 31 or 32 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 30, 31 or 32;

f) miR-374 comprising a sequence selected from the group consisting of SEQ ID No. 33, 34, 35, 36, 37 or 38 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 33, 34, 35, 36, 37 or 38;

g) miR-24 comprising a sequence selected from the group consisting of SEQ ID No. 39, 40, 41 or 42 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 39, 40, 41 or 42;

h) miR-483 comprising a sequence selected from the group consisting of SEQ ID No. 43 or 44 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 43 or 44;

i) miR-34 comprising a sequence selected from the group consisting of SEQ ID No. 45, 46, 47, 48, 49 or 50 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 45, 46, 47, 48, 49 or 50;

j) miR-20 comprising a sequence selected from the group consisting of SEQ ID No. 51, 52, 53 or 54 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 51, 52, 53 or 54; and

k) miR-15 comprising a sequence selected from the group consisting of SEQ ID No. 55, 56, 57 or 58 or a sequence having at least 90% nucleotide identity with one of SEQ ID No. 55, 56, 57 or 58.

4. The composition of claim 1, wherein the said precursor of miRNA consists of:

a) mir-92a-1 comprising the sequence SEQ ID No. 1, or a sequence having at least 90% nucleotide identity with SEQ ID No. 1;

b) mir-92a-2 comprising the sequence SEQ ID No. 2, or a sequence having at least 90% nucleotide identity with SEQ ID No. 2;

c) mir-92b comprising the sequence SEQ ID No. 3, or a sequence having at least 90% nucleotide identity with SEQ ID No. 3;

d) mir-17 comprising the sequence SEQ ID No. 4 or a sequence having at least 90% nucleotide identity with SEQ ID No. 4;

e) mir-503 comprising the sequence SEQ ID No. 5 or a sequence having at least 90% nucleotide identity with SEQ ID No. 5;

f) mir-16-1 comprising the sequence SEQ ID No. 6, or a sequence having at least 90% nucleotide identity with SEQ ID No. 6; and

g) mir-16-2 comprising the sequence SEQ ID No. 7, or a sequence having at least 90% nucleotide identity with SEQ ID No. 7

h) mir-374a comprising the sequence SEQ ID No. 8, or a sequence having at least 90% nucleotide identity with SEQ ID No. 8

i) mir-374b comprising the sequence SEQ ID No. 9, or a sequence having at least 90% nucleotide identity with SEQ ID No. 9

j) mir-374c comprising the sequence SEQ ID No. 10, or a sequence having at least 90% nucleotide identity with SEQ ID No. 10

k) mir-24-1 comprising the sequence SEQ ID No. 11, or a sequence having at least 90% nucleotide identity with SEQ ID No. 11

l) mir-24-2 comprising the sequence SEQ ID No. 12, or a sequence having at least 90% nucleotide identity with SEQ ID No. 12

m) mir-483 comprising the sequence SEQ ID No. 13, or a sequence having at least 90% nucleotide identity with SEQ ID No. 13

n) mir-34a comprising the sequence SEQ ID No. 14, or a sequence having at least 90% nucleotide identity with SEQ ID No. 14

o) mir-34b comprising the sequence SEQ ID No. 15, or a sequence having at least 90% nucleotide identity with SEQ ID No. 15

p) mir-34c comprising the sequence SEQ ID No. 16, or a sequence having at least 90% nucleotide identity with SEQ ID No. 16

q) mir-20a comprising the sequence SEQ ID No. 17, or a sequence having at least 90% nucleotide identity with SEQ ID No. 17

r) mir-20b comprising the sequence SEQ ID No. 18, or a sequence having at least 90% nucleotide identity with SEQ ID No. 18

s) mir-15a comprising the sequence SEQ ID No. 19, or a sequence having at least 90% nucleotide identity with SEQ ID No. 19, and

t) mir-15b comprising the sequence SEQ ID No. 20, or a sequence having at least 90% nucleotide identity with SEQ ID No. 20.

5. The composition of claims 1 to 4, wherein the said inhibitor of a miRNA is an oligonucleotide of 8-49 nucleotides in length having a sequence targeted to the said miRNA, or a precursor thereof.

6. The composition of claim 5, wherein the said oligonucleotide is an antisense oligonucleotide that is at least partially complementary to the sequence of the target miRNA, or a precursor thereof.

7. The composition of claim 6, wherein said antisense oligonucleotide is selected from the group consisting of a ribonucleotide, a deoxyribonucleotide, a small RNA, an antagomir, a LNA, a CDNA, a PNA, a morpholino oligonucleotide or a combination thereof.

8. The composition of claim 6, wherein said oligonucleotide consists of an antagomir.

9. The composition of claim 8, wherein said antagomir comprises a nucleotide sequence comprising at least 16 contiguous nucleotides complementary to the nucleotides of a sequence selected from the group consisting of SEQ ID No. 1 to 58.

10. The composition of claim 9, wherein said antagomir comprises the sequence SEQ ID No. 59 or 60 and modifications excluding base substitutions thereof, and fragments consisting of subsequences of SEQ ID NO: 59 or 60 of at least 8 contiguous nucleotides thereof.

11. The composition of claims 1 to 10, wherein said microspheres are presenting a diameter which does not exceed 25 μm.

12. The composition of claims 1 to 10, wherein at least 50% of said microspheres are presenting a diameter comprised between 5 and 20 μm, preferentially between 5 and 15 μm.

13. The composition of claims 1 to 12, wherein the microspheres are incorporating from 1% to 15 % w/w of inhibitor.

14. The composition of claims 1 to 12, wherein the microspheres are incorporating from 1% to 10 % w/w of inhibitor.

15. The composition of claims 1 to 14, wherein said microspheres are made of a polymer consisting of poly-d,l-lactide (PLA), the said polymer being optionally blended with one or more other polymers.

16. The composition of claims 1 to 14, wherein said microspheres are made of a copolymer consisting of poly-d,l-lactide-co-glycolide (PLGA), the said polymer being optionally blended with one or more other polymers.

17. The composition of claims 1 to 14, wherein said microspheres are made of a blend of polymers consisting of poly-d,l-lactide-co-glycolide (PLGA) and poly-d,l-lactide (PLA).

18. The composition of claim 16 or 17, wherein the ratio of lactide:glycolide in the PLGA polymer is comprised between 50:50 to 95:5 molar ratio.

19. The composition of claims 16 to 18, wherein the inherent viscosity of the polymer is comprised between 0.1 and 0.70 dl/g.

20. The composition of any one of the preceding claims for use in the treatment of the myocardial infarction.

21. The composition of claim 20 wherein said myocardial infarction consists of the acute myocardial infarction.

22. A method of reversing or preventing ventricular remodelling in a subject in need thereof comprising administering to said subject an effective amount of a composition according to one of the claims 1 to 19.

23. The method of claim 22, wherein said administration consists of an administration by intracoronary route.

24. A population of biodegradable and biocompatible microspheres for use in the treatment or prevention of myocardial infarction, wherein said microspheres:

- have an average diameter comprised between 5 and 15 µm;
- are made of poly-d,l-lactide-co-glycolide (PLGA) ; poly-d,l-lactide (PLA) or a blend thereof;
- are incorporating from 1% to 15% w/w of a therapeutic agent capable of preventing ventricular remodelling,

wherein said therapeutic agent consists of an inhibitor of a miRNA selected from the group consisting of miR-92 (including miR-92a-1, miR-92a-2 and miR-92b), miR-17, miR-503, miR-16 (including miR-16-1 and miR-16-2), miR-374 (including miR-374a, miR-374b and miR-374c), miR-24 (including miR-24-1 and miR-24-2), miR-483, miR-34 (including miR-34a, miR-34b and miR-34c), miR-20 (including miR-20a and miR-20b), miR-15 (including miR-15a and miR-15b) and more preferentially miR-92a, or a precursor thereof.

25. The microspheres of claim 24, wherein said inhibitor is an antagomir.

26. A kit comprising at least i) a composition according to claim 1 to 19 and/or microspheres according to claims 24 or 25 and ii) a syringe or vial or ampoule in which the composition is disposed.

27. The kit of claim 26, further comprising a solvent disposed in a solvent container.

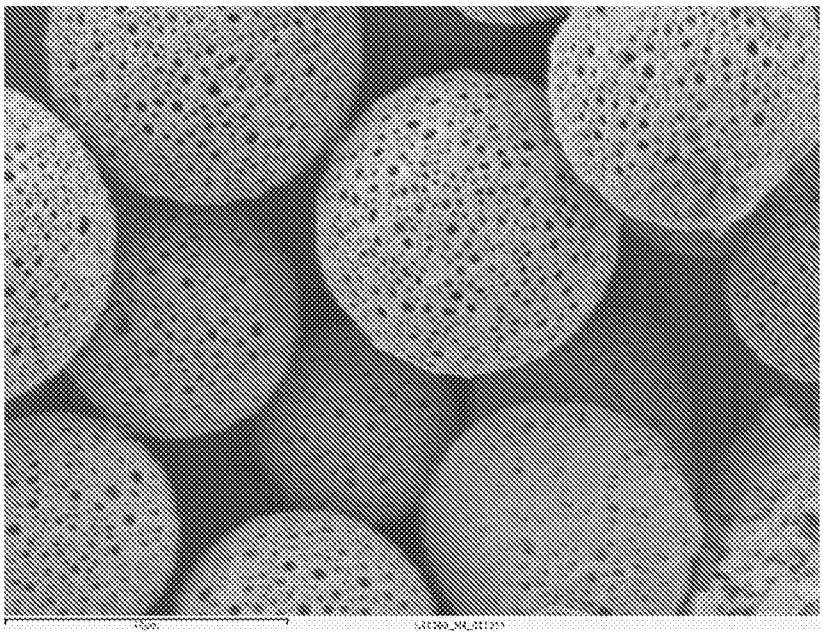

Figure 1

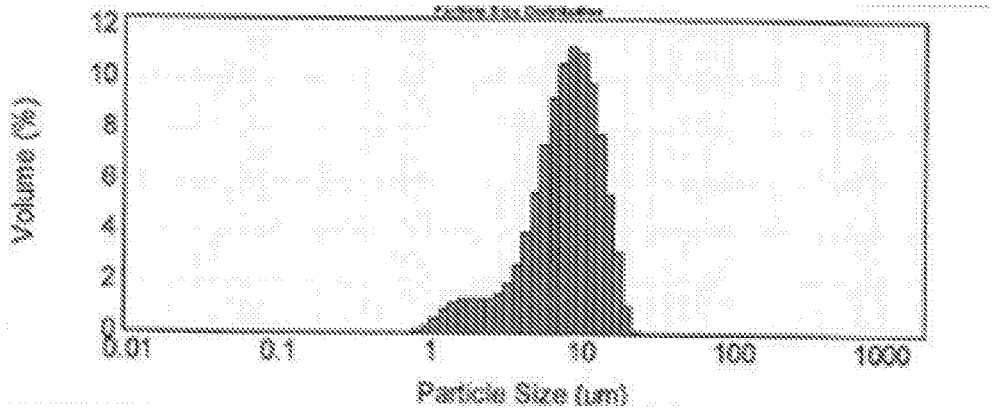

Figure 2

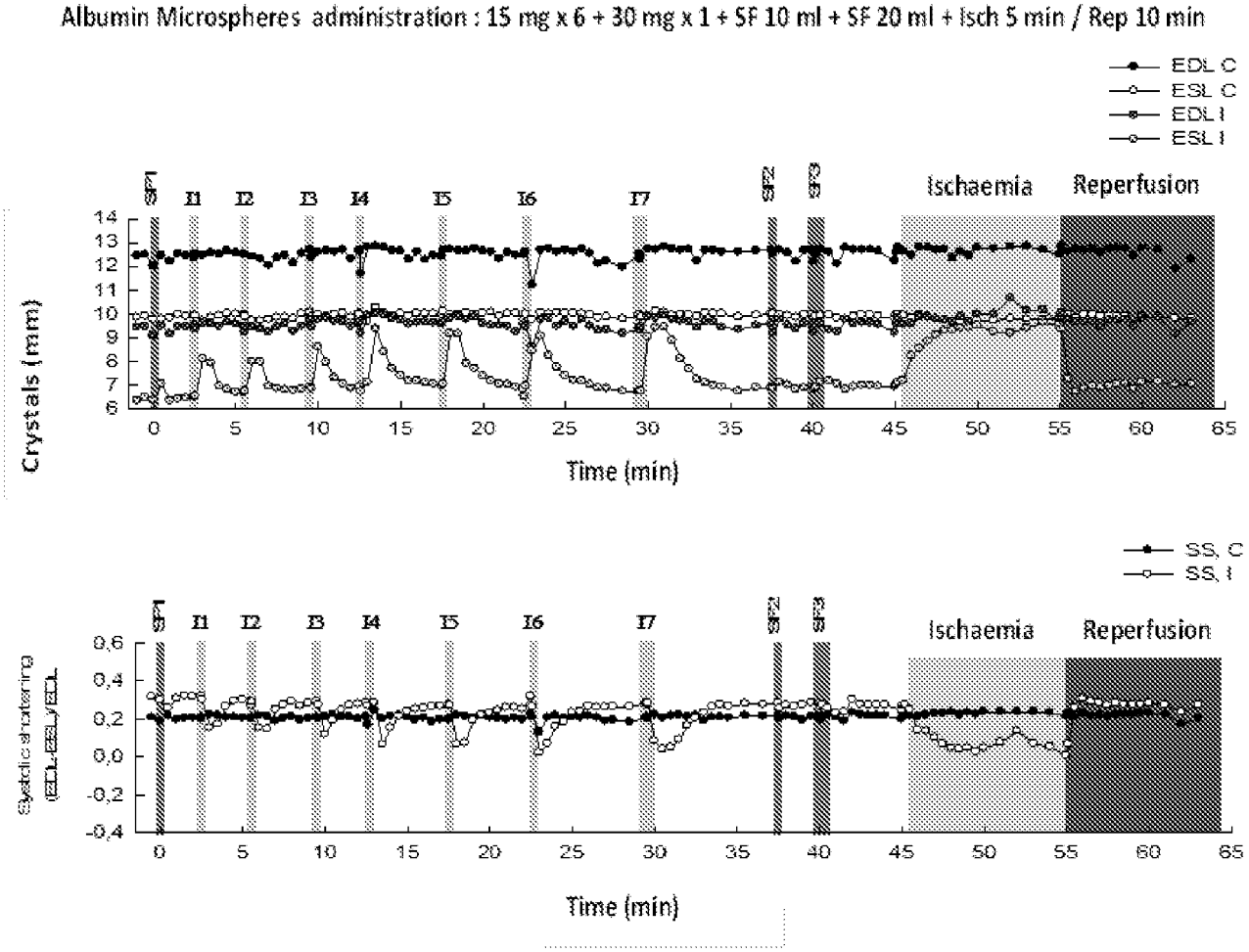
Albumin Microspheres administration : 15 mg x 6 + 30 mg x 1 + SF 10 ml + SF 20 ml + Isch 5 min / Rep 10 min
EDL C
ESL C
EDL I
ESL I
SF1
I1
I2
I3
I4
I5
I6
I7
SF2
SF3
Ischaemia
Reperfusion
Crystals (mm)
Time (min)
SS, C
SS, I
Systolic shortening (EDL-ESL)/EDL
Time (min)

Figure 3

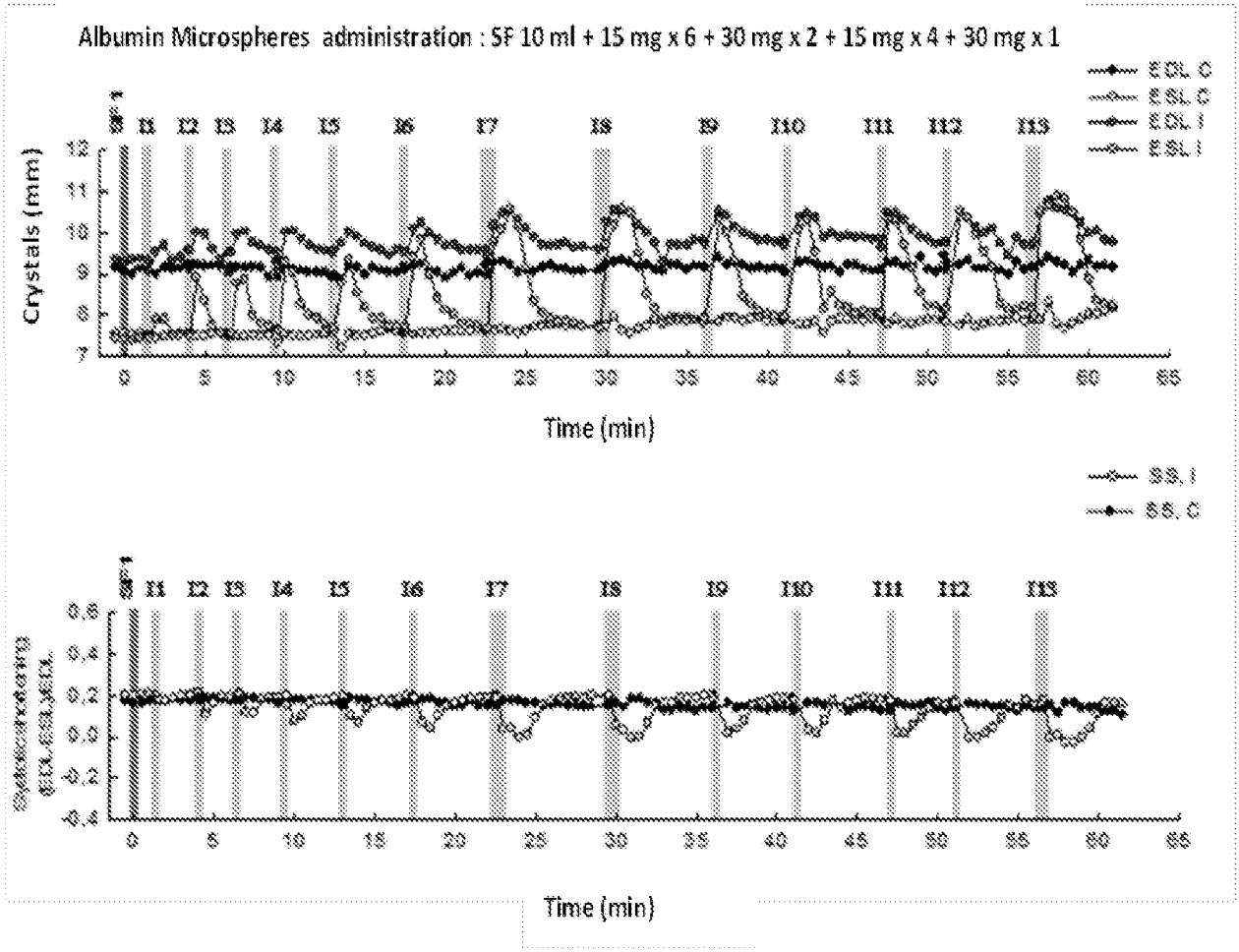
Albumin Microspheres administration : SF 10 ml + 15 mg x 6 + 30 mg x 2 + 15 mg x 4 + 30 mg x 1
Crystals (mm)
Time (min)
Time (min)

Figure 4

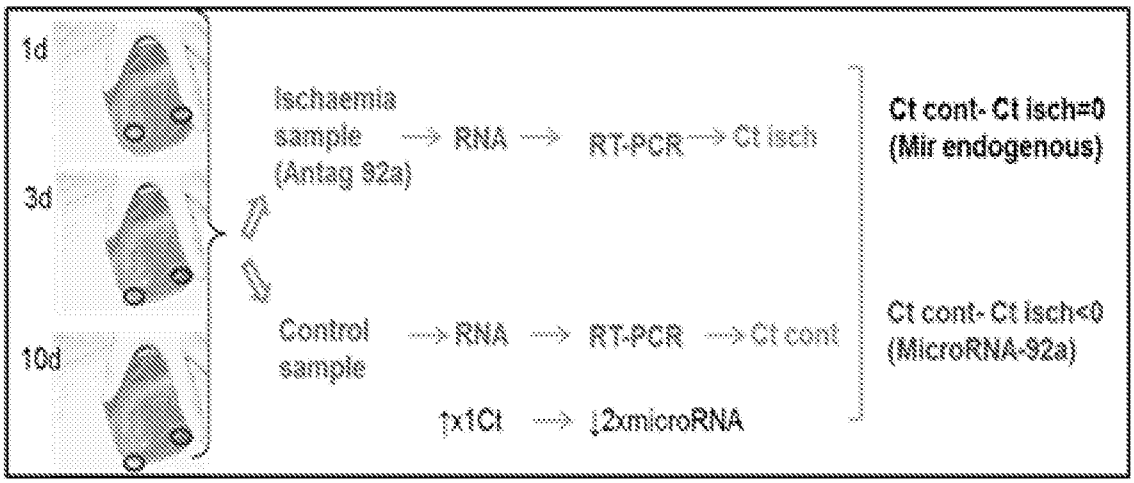
1d
3d
10d
Ischaemia sample (Antag 92a)
RNA
RT-PCR
Ct isch
Control sample
RNA
RT-PCR
Ct cont
↑x1Ct
↓2xmicroRNA
Ct cont- Ct isch=0 (Mir endogenous)
Ct cont- Ct isch<0 (MicroRNA-92a)

Figure 5

A **B**

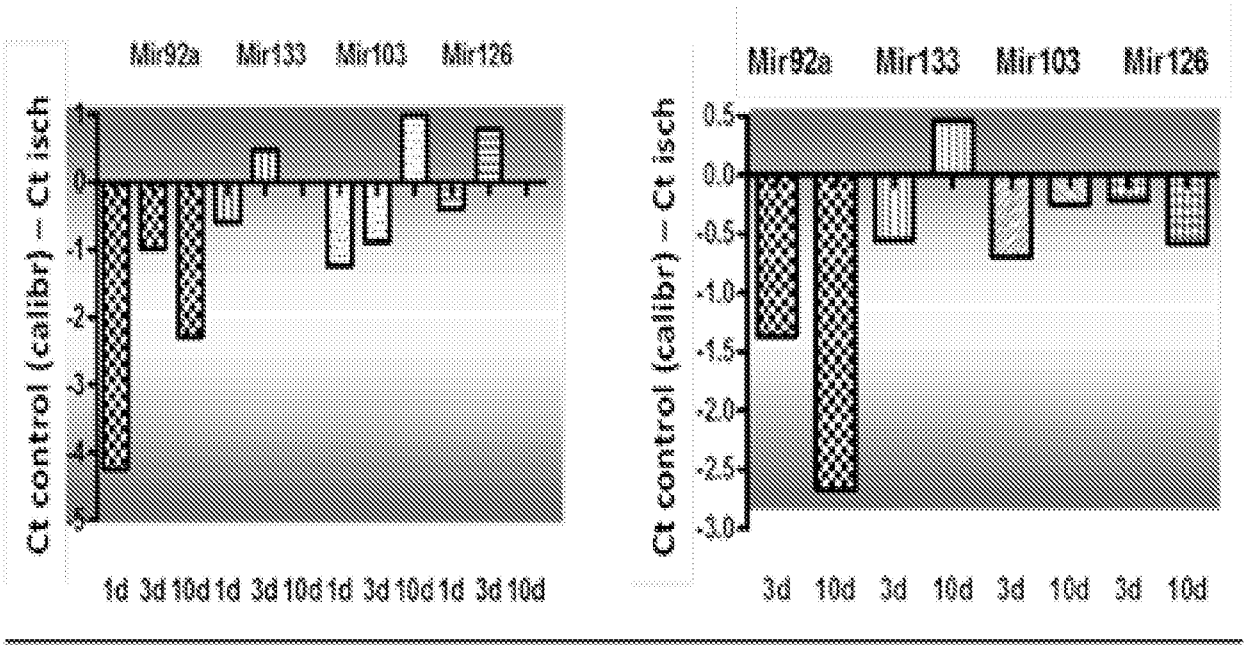
Mir92a
Mir133
Mir103
Mir126
Ct control (calibr) – Ct isch
1d 3d 10d 1d 3d 10d 1d 3d 10d 1d 3d 10d
3d 10d 3d 10d 3d 10d 3d 10d

Figure 6

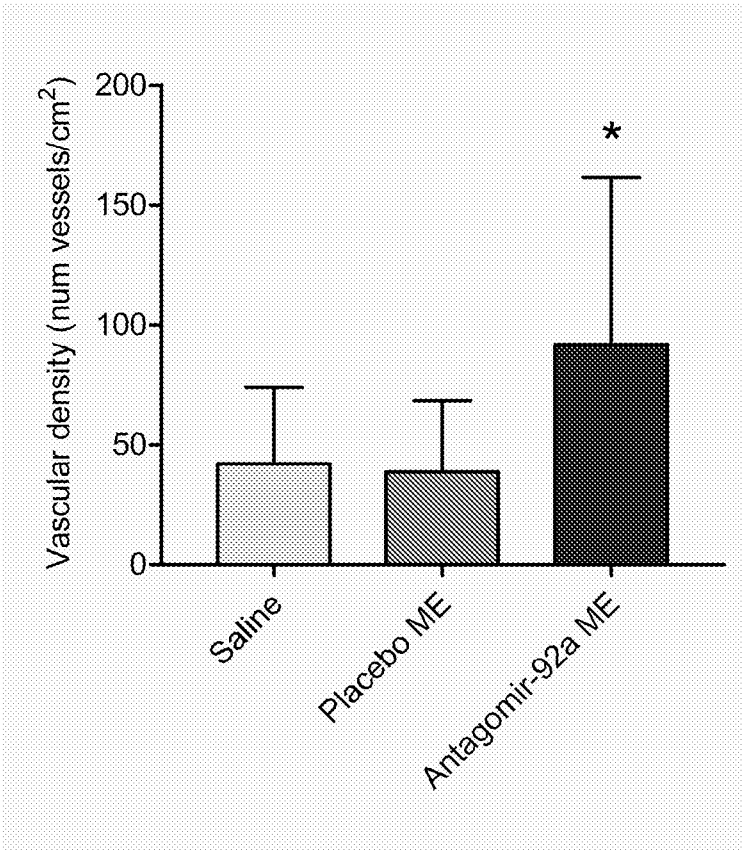
Vascular density (num vessels/cm2)
200
150
100
50
0
*
Saline
Placebo ME
Antagomir-92a ME

Figure 7

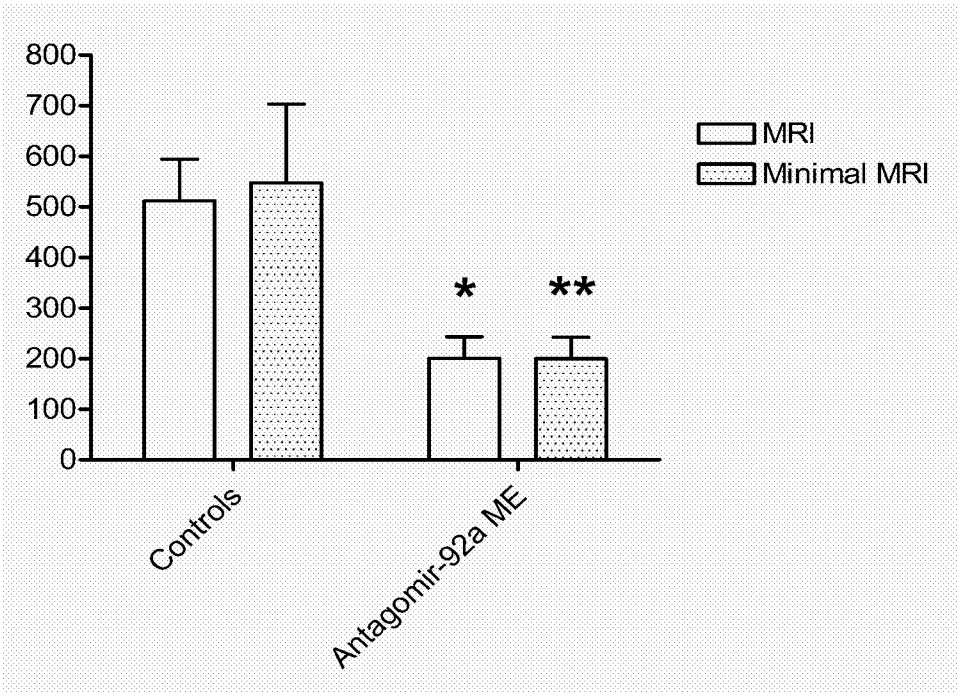

Figure 8

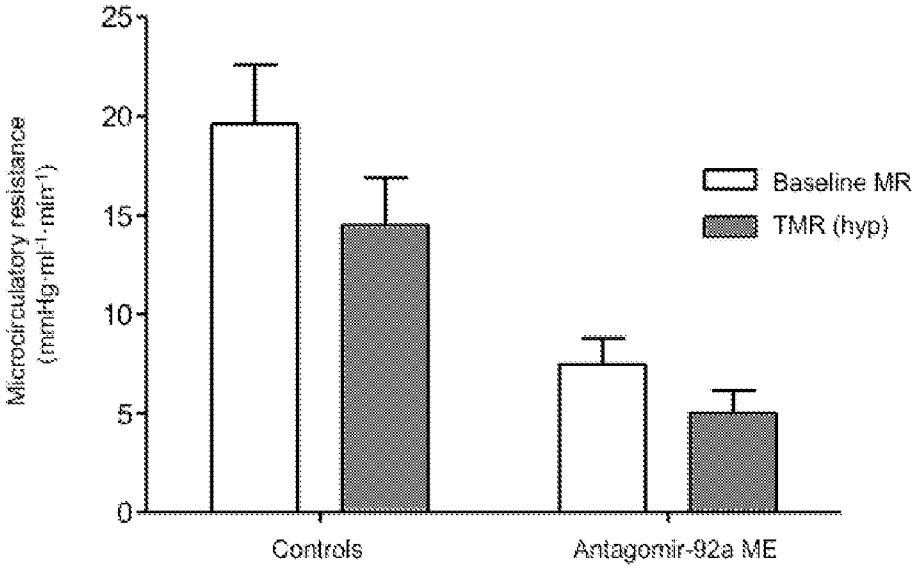

Figure 9

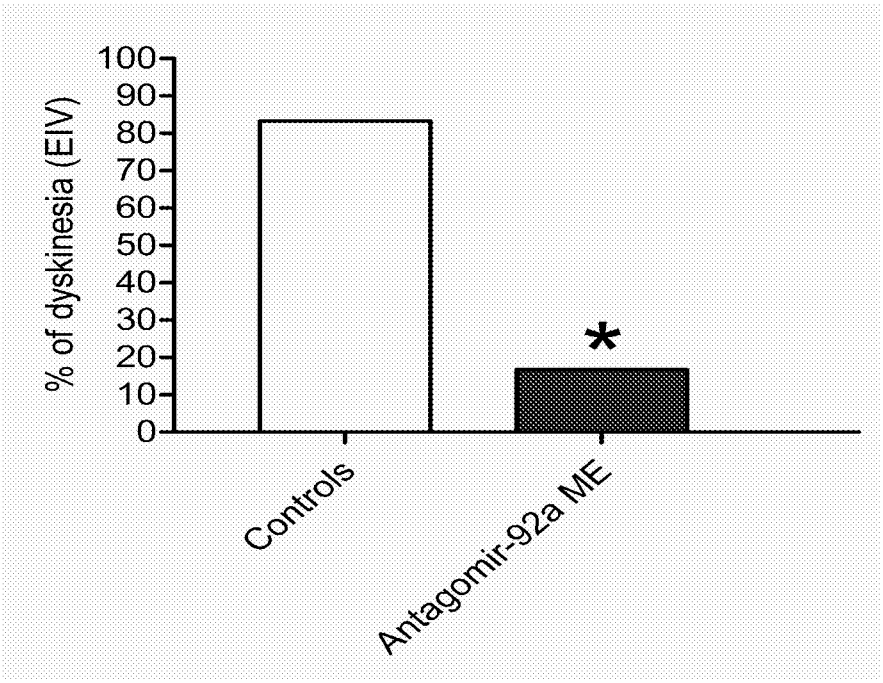

Figure 10

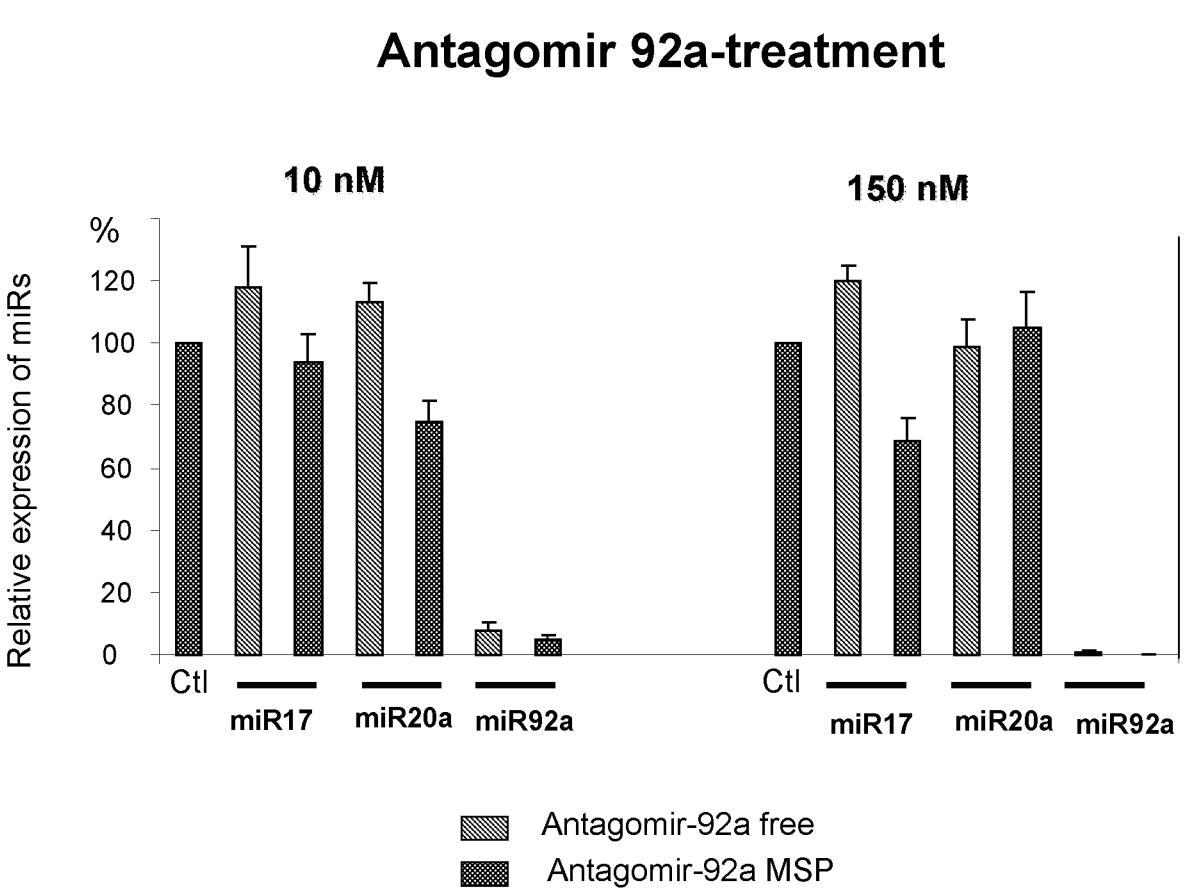

Figure 11

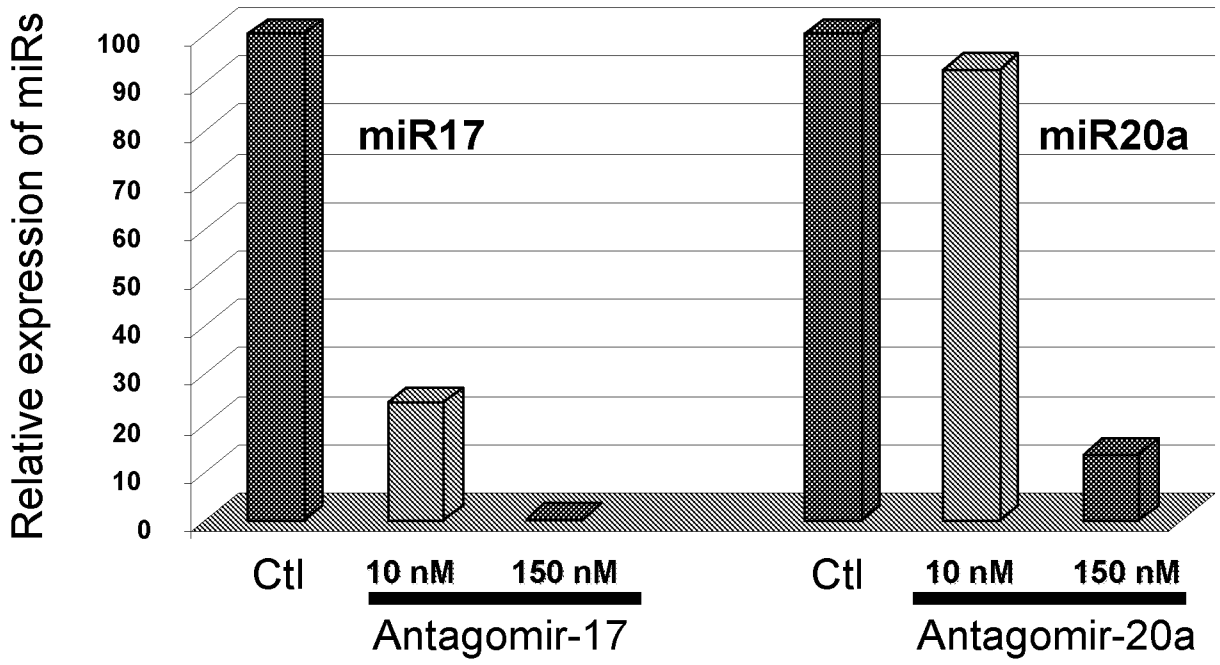
Relative expression of miRs
100
90
80
70
60
50
40
30
20
10
0
miR17
miR20a
Ctl
10 nM
150 nM
Antagomir-17
Ctl
10 nM
150 nM
Antagomir-20a

Figure 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/058500

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/712
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; November 2011 (2011-11), FUJITA MASANORI ET AL: "Inhibition of MicroRNA-92a Enhances Angiogenesis and Cardiomyocyte Regeneration Through Integrin a5-Dependent Accumulation of Stem Cells Into the Infarcted Myocardium", XP055116076, Database accession no. PREV201200224175 abstract -/--	1-10,20, 21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 April 2014	12/05/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Romano, Alper

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& CIRCULATION, vol. 124, no. 21, Suppl. S, November 2011 (2011-11), page A12590, SCIENTIFIC SESSIONS OF THE AMERICAN-HEART-ASSOCIATION/RESUSCITATION SCIENCE SYMPOSIUM; ORLANDO, FL, USA; NOVEMBER 12 -16, 2011 ISSN: 0009-7322(print) -----	
X,P	K Ablasser ET AL: "Cardiovascular biology 1053 P5665 ¦ BENCH Static, but not cyclic tensional forces on the heart increase anti-angiogenic sFlt-1 expression in endothelial cells and inhibit VEGFR-2 activation P5666 ¦ BENCH Intracoronary injection of encapsulated antagomir-92a promotes angiogenesis and prevents adverse", , 1 August 2013 (2013-08-01), XP055116081, Retrieved from the Internet: URL:http://eurheartj.oxfordjournals.org/content/34/suppl_1/P5666.full.pdf [retrieved on 2014-04-30] abstract -----	1-14,16, 20-25
Y	BONAUER ANGELIKA ET AL: "MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice", SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, WASHINGTON, DC; US, vol. 324, no. 5935, 26 June 2009 (2009-06-26), pages 1710-1713, XP002575234, ISSN: 0036-8075 the whole document -----	11-27
Y	D'ANGELO IVANA ET AL: "Improved delivery of angiogenesis inhibitors from PLGA:poloxamer blend micro- and nanoparticles.", JOURNAL OF MICROENCAPSULATION 2010, vol. 27, no. 1, 2010, pages 57-66, XP002700281, ISSN: 1464-5246 table 2 -----	11-27

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/058500

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PANYAM J ET AL: "Biodegradable nanoparticles for drug and gene delivery to cells and tissue", ADVANCED DRUG DELIVERY REVIEWS, ELSEVIER BV, AMSTERDAM, NL, vol. 55, no. 3, 24 February 2003 (2003-02-24), pages 329-347, XP008096954, ISSN: 0169-409X, DOI: 10.1016/S0169-409X(02)00228-4 the whole document -----	1-27
A	CLAUDIO IACONETTI ET AL: "Inhibition of miR-92a increases endothelial proliferation and migration in vitro as well as reduces neointimal proliferation in vivo after vascular injury", BASIC RESEARCH IN CARDIOLOGY, STEINKOPFF-VERLAG, DA, vol. 107, no. 5, 14 August 2012 (2012-08-14), pages 1-14, XP035112376, ISSN: 1435-1803, DOI: 10.1007/S00395-012-0296-Y the whole document -----	1-27
A	JAGAT R KANWAR ET AL: "Antiangiogenic therapy using nanotechnological-based delivery system", DRUG DISCOVERY TODAY, vol. 16, no. 5, 1 March 2011 (2011-03-01), pages 188-202, XP028162998, ISSN: 1359-6446, DOI: 10.1016/J.DRUDIS.2011.01.007 [retrieved on 2011-01-22] the whole document -----	1-27

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代理人 孟凡宏
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权利要求书3页 说明书29页
序列表14页 附图7页

(54)发明名称

包含包封的antagomir的组合物

(57)摘要

本发明涉及包含有效量的涉及血管生成的miRNA或其前体的至少一种抑制剂的组合物，其中将所述抑制剂微包封成聚合物生物可降解且生物相容的微球。本发明还涉及所述组合物用于预防或治疗心脏疾病，包括由缺血引起的心脏疾病的用途。

CN 105229151 A

1.一种组合物,包含有效量的涉及血管生成的miRNA或其前体的至少一种抑制剂,其中将所述抑制剂微包封成聚合物生物可降解且生物相容的微球。

2.权利要求1所述的组合物,其中所述miRNA选自miR-92(包括miR-92a-1、miR-92a-2和miR-92b)、miR-17、miR-503、miR-16(包括miR-16-1和miR-16-2)、miR-374(包括miR-374a、miR-374b和miR-374c)、miR-24(包括miR-24-1和miR-24-2)、miR-483、miR-34(包括miR-34a、miR-34b和miR-34c)、miR-20(包括miR-20a和miR-20b)、miR-15(包括miR-15a和miR-15b)。

3.权利要求1所述的组合物,其中所述miRNA由以下成熟miRNA组成:

a)包含选自SEQ ID NO:21、22或23的序列或与SEQ ID NO:21、22或23之一具有至少90%核苷酸一致性的序列的miR-92a;

b)包含选自SEQ ID NO:24或25的序列或与SEQ ID NO:24或25之一具有至少90%核苷酸一致性的序列的miR-92b;

c)包含选自SEQ ID NO:26或27的序列或与SEQ ID NO:26或27之一具有至少90%核苷酸一致性的序列的miR-17;

d)包含选自SEQ ID NO:28或29的序列或与SEQ ID NO:28或29之一具有至少90%核苷酸一致性的序列的miR-503;

e)包含选自SEQ ID NO:30、31或32的序列或与SEQ ID NO:30、31或32之一具有至少90%核苷酸一致性的序列的miR-16;

f)包含选自SEQ ID NO:33、34、35、36、37或38的序列或与SEQ ID NO:33、34、35、36、37或38之一具有至少90%核苷酸一致性的序列的miR-374;

g)包含选自SEQ ID NO:39、40、41或42的序列或与SEQ ID NO:39、40、41或42之一具有至少90%核苷酸一致性的序列的miR-24;

h)包含选自SEQ ID NO:43或44的序列或与SEQ ID NO:43或44之一具有至少90%核苷酸一致性的序列的miR-483;

i)包含选自SEQ ID NO:45、46、47、48、49或50的序列或与SEQ ID NO:45、46、47、48、49或50之一具有至少90%核苷酸一致性的序列的miR-34;

j)包含选自SEQ ID NO:51、52、53或54的序列或与SEQ ID NO:51、52、53或54之一具有至少90%核苷酸一致性的序列的miR-20;和

k)包含选自SEQ ID NO:55、56、57或58的序列或与SEQ ID NO:55、56、57或58之一具有至少90%核苷酸一致性的序列的miR-15。

4.权利要求1所述的组合物,其中所述miRNA前体由以下组成:

a)包含SEQ ID NO:1的序列或与SEQ ID NO:1具有至少90%核苷酸一致性的序列的mir-92a-1;

b)包含SEQ ID NO:2的序列或与SEQ ID NO:2具有至少90%核苷酸一致性的序列的mir-92a-2;

c)包含SEQ ID NO:3的序列或与SEQ ID NO:3具有至少90%核苷酸一致性的序列的mir-92b;

d)包含SEQ ID NO:4的序列或与SEQ ID NO:4具有至少90%核苷酸一致性的序列的mir-17;

e) 包含 SEQ ID NO:5 的序列或与 SEQ ID NO:5 具有至少 90％核苷酸一致性的序列的 mir-503；

f) 包含 SEQ ID NO:6 的序列或与 SEQ ID NO:6 具有至少 90％核苷酸一致性的序列的 mir-16-1；

g) 包含 SEQ ID NO:7 的序列或与 SEQ ID NO:7 具有至少 90％核苷酸一致性的序列的 mir-16-2；

h) 包含 SEQ ID NO:8 的序列或与 SEQ ID NO:8 具有至少 90％核苷酸一致性的序列的 mir-374a；

i) 包含 SEQ ID NO:9 的序列或与 SEQ ID NO:9 具有至少 90％核苷酸一致性的序列的 mir-374b；

j) 包含 SEQ ID NO:10 的序列或与 SEQ ID NO:10 具有至少 90％核苷酸一致性的序列的 mir-374c；

k) 包含 SEQ ID NO:11 的序列或与 SEQ ID NO:11 具有至少 90％核苷酸一致性的序列的 mir-24-1；

l) 包含 SEQ ID NO:12 的序列或与 SEQ ID NO:12 具有至少 90％核苷酸一致性的序列的 mir-24-2；

m) 包含 SEQ ID NO:13 的序列或与 SEQ ID NO:13 具有至少 90％核苷酸一致性的序列的 mir-483；

n) 包含 SEQ ID NO:14 的序列或与 SEQ ID NO:14 具有至少 90％核苷酸一致性的序列的 mir-34a；

o) 包含 SEQ ID NO:15 的序列或与 SEQ ID NO:15 具有至少 90％核苷酸一致性的序列的 mir-34b；

p) 包含 SEQ ID NO:16 的序列或与 SEQ ID NO:16 具有至少 90％核苷酸一致性的序列的 mir-34c；

q) 包含 SEQ ID NO:17 的序列或与 SEQ ID NO:17 具有至少 90％核苷酸一致性的序列的 mir-20a；

r) 包含 SEQ ID NO:18 的序列或与 SEQ ID NO:18 具有至少 90％核苷酸一致性的序列的 mir-20b；

s) 包含 SEQ ID NO:19 的序列或与 SEQ ID NO:19 具有至少 90％核苷酸一致性的序列的 mir-15a；和

t) 包含 SEQ ID NO:20 的序列或与 SEQ ID NO:20 具有至少 90％核苷酸一致性的序列的 mir-15b。

5. 权利要求 1-4 所述的组合物，其中所述 miRNA 抑制剂是长度为 8-49 个核苷酸的具有靶向所述 miRNA 或其前体的序列的寡核苷酸。

6. 权利要求 5 所述的组合物，其中所述寡核苷酸是与所述靶 miRNA 或其前体至少部分互补的反义寡核苷酸。

7. 权利要求 6 所述的组合物，其中所述反义寡核苷酸选自核糖核苷酸、脱氧核糖核苷酸、小 RNA、antagomir、LNA、CDNA、PNA、吗啉代寡核苷酸或它们的组合。

8. 权利要求 6 所述的组合物，其中所述寡核苷酸由 antagomir 组成。

9. 权利要求 8 所述的组合物，其中所述 antagomir 包含核苷酸序列，所述核苷酸序列包含至少 16 个与选自 SEQ ID NO :1-58 的序列的核苷酸互补的连续核苷酸。

10. 权利要求 9 所述的组合物，其中所述 antagomir 包含序列 SEQ ID NO :59 或 60 及其除碱基替换之外的修饰，以及由至少 8 个连续核苷酸的 SEQ ID NO :59 或 60 的子序列组成的片段。

11. 权利要求 1-10 所述的组合物，其中所述微球的直径不超过 25 μm。

12. 权利要求 1-10 所述的组合物，其中至少 50％的所述微球的直径为 5-20 μm，优选 5-15 μm。

13. 权利要求 1-12 所述的组合物，其中所述微球包含 1％ -15％ w/w 的抑制剂。

14. 权利要求 1-12 所述的组合物，其中所述微球包含 1％ -10％ w/w 的抑制剂。

15. 权利要求 1-14 所述的组合物，其中所述微球用由聚 -d,l- 丙交酯 (PLA) 组成的聚合物制成，所述聚合物任选地与一种或多种其他聚合物共混。

16. 权利要求 1-14 所述的组合物，其中所述微球用由聚 -d,l- 丙交酯 - 共 - 乙交酯 (PLGA) 组成的共聚物制成，所述聚合物任选地与一种或多种其他聚合物共混。

17. 权利要求 1-14 所述的组合物，其中所述微球用由聚 -d,l- 丙交酯 - 共 - 乙交酯 (PLGA) 和聚 -d,l- 丙交酯 (PLA) 组成的聚合物共混物制成。

18. 权利要求 16 或 17 所述的组合物，其中 PLGA 聚合物中丙交酯 :乙交酯的比例为 50:50 至 95:5 摩尔比。

19. 权利要求 16-18 所述的组合物，其中所述聚合物的固有粘度为 0.1-0.70dl/g。

20. 前述权利要求任一项所述的组合物用于治疗心肌梗死的用途。

21. 权利要求 20 所述的组合物，其中所述心肌梗死是急性心肌梗死。

22. 在需要治疗的受试者中逆转或预防心室重构的方法，包括向所述受试者施用有效量的根据权利要求 1-19 任一项所述的组合物。

23. 权利要求 22 所述的方法，其中所述施用是通过冠状动脉内途径施用。

24. 生物可降解且生物相容的微球群体用于治疗或预防心肌梗死的用途，其中所述微球 :

- 平均直径为 5-15 μm ;
- 由聚 -d,l- 丙交酯 - 乙交酯共聚物 (PLGA)、聚 -d,l- 丙交酯 (PLA) 或它们的共混物制成 ;
- 包含 1％ -15％ w/w 的能够预防心室重构的治疗剂 ;

其中所述治疗剂由选自以下的 miRNA 或其前体的抑制剂组成 :miR-92(包括 miR-92a-1、miR-92a-2 和 miR-92b)、miR-17、miR-503、miR-16(包括 miR-16-1 和 miR-16-2)、miR-374(包括 miR-374a、miR-374b 和 miR-374c)、miR-24(包括 miR-24-1 和 miR-24-2)、miR-483、miR-34(包括 miR-34a、miR-34b 和 miR-34c)、miR-20(包括 miR-20a 和 miR-20b)、miR-15(包括 miR-15a 和 miR-15b)，优选 miR-92a。

25. 权利要求 24 所述的微球，其中所述抑制剂是 antagomir。

26. 一种试剂盒，包括至少 i) 根据权利要求 1-19 所述的组合物和 / 或根据权利要求 24 或 25 所述的微球 ;ii) 置放所述组合物的注射器或小瓶或安瓿。

27. 权利要求 26 所述的试剂盒，进一步包括置于溶剂容器中的溶剂。

包含包封的 antagomir 的组合物

技术领域

[0001] 本发明涉及旨在预防或治疗心脏疾病，包括心脏缺血性疾病的药物制剂的领域。

背景技术

[0002] 急性心肌梗死 (AMI)，也称为心肌梗死并通常被称为心脏病发作，代表世界上大多数工业化国家的一个主要健康风险，并且仍然是世界范围内发病率和死亡率的主要原因。

[0003] 通常，AMI 是由突然且持续缺乏流向心脏组织的血液造成，这一般是冠状动脉狭窄或闭塞的结果。没有充分的血液供给，所述组织变得缺血，造成心肌细胞（心脏肌肉细胞）和血管结构的死亡。

[0004] 近几十年来在治疗 AMI 中有很多进展，主要涉及与药物疗法相结合的冠状动脉再灌注。再灌注疗法已经通过减少梗死面积和改善短期和长期的发病率和死亡率而成功改变 AMI 的自然进展。然而，使用目前可获得的两种再灌注策略有实质性的限制 :纤维蛋白溶解导致低程度的冠状动脉通透性，在 AMI 进化的开始几小时期间不能频繁应用初期血管成形术。此外，在一些插管的患者中，除了正常的心外膜血流，出现“无复流”现象或缺乏微血管再灌注，导致不良功能性结果。这意味着再灌注疗法不能预防心肌的有害重构（胶原蛋白疤痕形成的复杂的内在修复过程，导致心室扩张、收缩功能障碍和随后的心脏衰竭）的发生。它在大约 30％ AMI 中的发生主要与仍知之甚少的较大的梗死面积、微血管阻塞及不良修复反应有关。

[0005] 因为修复性纤维化和缺血组织的功能恢复依赖于建立供应氧合血的网络，已经做出努力以通过在愈合区域诱导新血管形成来改善血管床，从而在原位实现将损伤区域直接转变为功能性组织。

[0006] 血管生成的治疗性诱导可以减轻这种现象的发生。尽管如此，若干年静脉促血管生成因子的临床试验结果并不令人满意。不被理论束缚，认为造成这种失败的原因之一是静脉途径不能够在严重受损的心脏条件下到达并在靶组织中维持有效的药物浓度，从而促进并维持功能性的血管网络。

[0007] 为了解决这个问题并预防 AMI 后的心功能不全，在过去十年内开始了再生心肌和血管可能性的研究。施用多能祖细胞和灌注血管生长因子的初始研究显示有前途的结果，但转到临床设置则显示这些治疗不能以足够恢复被破坏的心肌的方式再生新血管。

[0008] 今天，血管生成和用细胞治疗的受损心脏的再增殖以及特定的药物和再同步化治疗能够减缓这种现象的进展，但预防它的发生对于心脏医学仍然是有疑问的挑战。因此，需要预防有害的左心室重构的新的治疗策略。

[0009] 发明简述

[0010] 本发明涉及通过施用通过调节 microRNA 的活性或表达来逆转或预防心室重构的组合物治疗心脏疾病，优选缺血性疾病。更具体地，本发明提供包含涉及血管生成的 microRNA 的抑制剂的组合物，所述抑制剂优选包含于能够将所述抑制剂局部释放至缺血靶组织的新的递送介质中。此外，本发明提供逆转或预防心室重构的方法和用于所述方法的

试剂盒。

[0011] 本发明涉及一种组合物，其包含有效量的至少一种涉及血管生成的 miRNA 或其前体的抑制剂，其中将所述抑制剂微包封成聚合的生物可降解且生物相容的微球。

[0012] 在上述组合物的一些实施方案中，所述 miRNA 选自包含 miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和 miR-15b）的家族。

[0013] 在上述组合物的一些实施方案中，所述 miRNA 的抑制剂是长度为 8-49 个核苷酸的具有靶向所述 miRNA 或其前体的序列的寡核苷酸。在某些实施方案中，所述寡核苷酸是与所述靶 miRNA 或其前体至少部分互补的反义寡核苷酸。所述反义寡核苷酸选自核糖核苷酸、脱氧核糖核苷酸、小 RNA、antagomir、LNA、CDNA、PNA、吗啉代寡核苷酸或它们的组合。

[0014] 在上述组合物的一些实施方案中，所述 miRNA 的抑制剂由 antagomir 组成，优选由包含核苷酸序列的 antagomir 组成，所述核苷酸序列包含至少 16 个与选自本文所述的 SEQ ID NO：1-58 的序列的核苷酸互补的连续核苷酸。

[0015] 在上述组合物的一些实施方案中，所述 miRNA 的抑制剂由包含序列 SEQ ID NO：59 或 60 和其除碱基替换之外的修饰，及其片段的 antagomir 组成，所述片段由 SEQ ID NO：59 或 60 的至少 8 个连续核苷酸的子序列组成。

[0016] 在上述组合物的一些实施方案中，所述微球的直径不超过 25μm，包括直径为 5-20μm，优选 5-15μm 的微球。

[0017] 在上述组合物的一些实施方案中，所述微球由聚 -d,l- 丙交酯 (PLA) 组成的聚合物制成，所述聚合物任选地与一种或多种其他聚合物混合。

[0018] 本发明还涉及逆转或预防需要其的受试者心室重构的方法，包括向所述受试者施用有效量的上述组合物。

[0019] 本发明还涉及生物可降解且生物相容的微球群体用于治疗或预防心肌梗死的用途，其中所述微球：

[0020] - 平均直径为 5-20μm，优选 5-15μm；

[0021] - 由聚 -d,l- 丙交酯 - 乙交酯共聚物 (PLGA)；聚 -d,l- 丙交酯 (PLA) 或它们的混合物制成；

[0022] - 包含 1％ -15％ w/w 的能够预防心室重构的治疗剂；

[0023] 其中所述治疗剂由选自以下的 miRNA 或其前体的抑制剂组成：miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和 miR-15b），优选 miR-92a。

附图说明

[0024] 图 1 显示通过扫描电镜测定的 antagomir-92a-PLGA 微球的形态。

[0025] 图 2 显示通过激光散射测定的 antagomir-92a-PLGA 微球的尺寸分布。

[0026] 图 3 显示通过重复注射微球直到 120mg 对血液动力学和左心室收缩性的作用。

[0027] 图 4 显示通过重复注射微球直到 240mg 对血液动力学和左心室收缩性的作用。

[0028] 图 5 显示单次冠状动脉内注射根据本发明的微球的分子作用的研究方案。

[0029] 图 6 显示本发明的微球对 miR-92a 的抑制特异性。

[0030] 图 7 显示包封的 antagomir-92a 诱导梗死组织中的血管生成。通过将血管总数除以总的梗死面积计算梗死区的血管密度。N = 20.*P<0.01。

[0031] 图 8 :间接测量。AMI 后一个月,在用包封的 antagomir-92a、安慰剂或盐水处理的小猪的梗死相关动脉中测量基础和最小的微血管阻力指数。(a) 用血压 (mmHg) 乘以冠脉流量 (ml/min) 来计算基础微血管阻力指数 (MRI),所述血压用位于顶端 LAD(Pd) 的压力线测量,和所述冠脉流量用位于 LAD 远段的感应器定量。用在最大充血测量的相同参数计算最小微血管阻力指数 (MRI_{hyp}),所述最大充血通过向股静脉 12F 鞘静脉输注 140mg/min 的腺苷来实现。n = 12*p<0.01**p = 0.05。(b) 坏死区中血管总数和 MRI 之间的关系。R^2 0.41,p = 0.02,n = 12。(c) 坏死区中血管总数和最小 MRI 之间的关系。R^2 0.27,p = 0.08,n = 12。

[0032] 图 9 :直接测量。AMI 后一个月,在用包封的 antagomir-92a 处理和不处理的小猪的梗死相关动脉中测量基线和实际微循环阻力。(a) 用血压 (mmHg) 除以冠脉流量 (ml/min) 来计算基线微循环阻力 (基线 MR),所述血压用位于顶端 LAD(Pd) 的压力线测量,和所述冠脉血流量用位于 LAD 远段的感应器定量。处理组的基线 MR 显著低于对照 (7.47±1.33vs 19.62±2.98,p = 0.005)。n = 13。用在最大充血测量的相同参数计算实际微循环阻力 (TMR(hyp)),所述最大充血通过向股静脉 12F 鞘静脉输注 140mg/min 的腺苷来实现。在处理组中观察到显著低于对照的 TMR(hyp)(5.0±1.15vs 14.49±2.4,p = 0.006)。n = 13。(b) 所有坏死区中血管密度与基线 MR 之间的关系。R^2= 0.35,P = 0.033,n = 13。(c) 所有坏死区中血管密度与 TMR(hyp) 之间的关系。R^2= 0.31,P = 0.047,n = 13。

[0033] 图 10 :AMI 后一个月,用对分配处理盲法的超声心动图评估隔顶运动障碍 (septoapical diskynesia) 的存在。显示了处理和未处理动物中患有隔顶运动障碍动物的百分比 (83.3% vs 16.7% ,p = 0.03)。n = 20。

[0034] 图 11 :体外评估包封的 antagomir 92a 和未包封的 antagomir 92a 对 miR92a 表达的作用。

[0035] 图 12 :包封的 antagomir 17 和 20a 对它们各自 miRNA 的作用。

[0036] 发明详述

[0037] 以下给出由本发明涵盖的所有实施方案的描述相关的一些定义。

[0038] MicroRNA(MiR) 是作为生物过程关键调节器的小的非编码 RNA。

[0039] “MicroRNA”、“miRNA”或“miR”是指长度为约 18 至约 25 个核苷酸的非编码 RNA。这些 miR 可以来自多种来源,包括 :编码 miRNA 的单个基因,来自蛋白编码基因的内含子,或来自通常编码多个密切相关的 microRNA 的多顺反子的转录物。

[0040] 目前的知识显示,miRNA 由 RNA 聚合酶 II(pol II) 或 RNA 聚合酶 III(pol III) 转录,且来自一般为几千个碱基长的称为初级 miRNA 转录物 (pri-miRNA) 的初始转录物。pri-miRNA 在细胞核中被 RNase Drosha 加工成约 70-100 个核苷酸的发夹型前体 (pre-miRNA)。被运输到细胞质后,发夹 pre-miRNA 进一步被 Dicer 加工以产生双链

microRNA ;然后被称为成熟 microRNA 的其中一条链（有时两条链均可使用）被整合到 RNA 诱导的沉默复合物 (RISC)，其中它与它的靶 mRNA 通过碱基对互补相关联。在相对罕见情况下，即当 miRNA 碱基对与信使 RNA(mRNA) 靶标完全配对时，它促使 mRNA 降解。更普遍的，microRNA 与靶 mRNA 形成不完美的异源双链体，影响 mRNA 的稳定性或抑制 mRNA 的翻译。

[0041] “茎环序列”是指具有发夹结构且包含成熟 miRNA 序列的 RNA。pre-miRNA 序列和茎环序列可以重叠。茎环序列的实例可以在名为 miRBase 的 microRNA 数据库中发现。

[0042] “microRNA 前体”是指来源于基因组 DNA 且包含含有一个或多个 microRNA 序列的非编码结构化 RNA 的转录物。例如，在某些实施方案中，microRNA 前体是 pre-miRNA。在某些实施方案中，microRNA 前体是 pri-miRNA。

[0043] 以下说明书将遵循标准命名系统，其中没有大写的“mir-X”是指 pre-miRNA，而大写的“miR-X”是指成熟形式。当两个成熟 microRNA 来自相同 pre-miRNA 的不同臂，它们分别被加以后缀 -3p 或 -5p。当相对表达水平已知时，名称后面的星号表示 microRNA 相对于发夹另一条臂的 microRNA 以低水平表达。

[0044] 在以下说明书中，除非另有说明，表述 miR-X（如果有的话）用来指包括 -3p 和 -5p 两种形式的成熟 miRNA。

[0045] 为了避免疑问，在本说明书中，表述 microRNA、miRNA 和 MiR 是指相同产物。

[0046] 因此，在本发明上下文中，目的是用特别关注的涉及血管生成的 microRNA 调控血管生成或血管生成过程。因此，本发明的目的是调控属于涉及血管生成调控的 microRNA 家族的至少一种 microRNA。

[0047] 表述“microRNA 家族”意欲是指具有由调控血管生成或血管生成过程组成的相关功能的一组 microRNA。更具体地且非限制性地，所述 microRNA 选自显示出抗血管生成活性的 microRNA。

[0048] 更具体地，且非限制性地，这种 microRNA 选自 miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和 miR-15b），或其前体。对血管生成具有调控性质，优选拮抗性的任何 miRNA 应当被认为是该 microRNA 家族的一部分，这对于本领域技术人员而言是显而易见的。

[0049] 为了避免疑问，除非另有说明，以下说明书中的表述 microRNA 将是指已经从它的前体切割的成熟或经加工的 RNA。对于非成熟形式，将使用表述“前体”或“microRNA 前体”。

[0050] 下表 1 将本说明书涵盖的 microRNA 前体的不同序列进行了重新分组。

[0051]

SEQ ID NO:	名称	序列
1	mir-92a-1	CUUUCUACACAGGUUGGGAUCGGUUGCAAUGCUGUGUUUCUGUAUGGUAUUGCACUUGUCCCGGCCUGUUGAGUUUGG
2	mir-92a-2	UCAUCCCUGGGUGGGGAUUUGUUGCAUUACUUGUGUUCUAUAUAAAGUAUUGCACUUGUCCCGGCCUGUGGAAGA
3	mir-92b	CGGGCCCCGGGCGGGCGGGAGGGACGGGACGCGGUGCAGUGUUGUUUUUUCCCCCGCCAAUAUUGCACUCGUCCCGGCCUCCGGCCCCCCCGGCCC
4	mir-17	GUCAGAAUAAUGUCAAAGUGCUUACAGUGCAGGUAGUGAUAUGUGCAUCUACUGCAGUGAAGGCACUUGUAGCAUUAUGGUGAC
5	mir-503	UGCCCUAGCAGCGGGAACAGUUCUGCAGUGAGCGAUCGGUGCUCUGGGGUAUUGUUUCCGCUGCCAGGGUA
6	mir-16-1	GUCAGCAGUGCCUUAGCAGCACGUAAAUAUUGGCGUUAAGAUUCUAAAAUUAUCUCCAGUAUUAACUGUGCUGCUGAAGUAAGGUUGAC
7	mir-16-2	GUUCCACUCUAGCAGCACGUAAAUAUUGGCGUAGUGAAAUAUAUAUUAAACACCAAUAUUACUGUGCUGCUUUAGUGUGAC
8	mir-374a	UACAUCGGCCAUUAUAAUACAACCUGAUAAGUGUUAUAGCACUUAUCAGAUUGUAUUGUAAUUGUCUGUGUA
9	mir-374b	ACUCGGAUGGAUAUAAUACAACCUGCUAAGUGUCCUAGCACUUAGCAGGUUGUAUUAUCAUUGUCCGUGUCU
10	mir-374c	ACACGGACAAUGAUAAUACAACCUGCUAAGUGCUAGGACACUUAGCAGGUUGUAUUAUAUCCAUCCGAGU
11	mir-24-1	CUCCGGUGCCUACUGAGCUGAUAUCAGUUCUCAUUUUACACACUGGCUCAGUUCAGCAGGAACAGGAG
12	mir-24-2	CUCUGCCUCCCGUGCCUACUGAGCUGAAACACAGUUGGUUUGUGUACACUGGCUCAGUUCAGCAGGAACAGGG

[0052]

13	mir-483	GAGGGGGAAGACGGGAGGAAAGAAGGGAGUGGUUCCAUCACGCCUCCUCACUCCUCUCCUCCCGUCUUCUCCUCUC
14	mir-34a	GGCCAGCUGUGAGUGUUUCUUUGGCAGUGUCUUAGCUGGUUGUUGUGAGCAAUAGUAAGGAAGCAAUCAGCAAGUAUACUGCCCUAGAAGUGCUGCACGUUGUGGGGCCC
15	mir-34b	GUGCUCGGUUUGUAGGCAGUGUCAUUAGCUGAUUGUACUGUGGUGGUUACAAUCACUAACUCCACUGCCAUCAAAACAAGGCAC
16	mir-34c	AGUCUAGUUACUAGGCAGUGUAGUUAGCUGAUUGCUAAUAGUACCAAUCACUAACCACACGGCCAGGUAAAAAGAUU
17	mir-20a	GUAGCACUAAAGUGCUUAUAGUGCAGGUAGUGUUUAGUUAUCUACUGCAUUAUGAGCACUUAAAGUACUGC
18	mir-20b	AGUACCAAAGUGCUCAUAGUGCAGGUAGUUUUGGCAUGACUCUACUGUAGUAUGGGCACUUCCAGUACU
19	mir-15a	CCUUGGAGUAAAGUAGCAGCACAUAAUGGUUUGUGGAUUUUGAAAAGGUGCAGGCCAUAUUGUGCUGCCUCAAAAAUACAAGG
20	mir-15b	UUGAGGCCUUAAAGUACUGUAGCAGCACAUCAUGGUUUACAUGCUACAGUCAAGAUGCGAAUCAUUAUUUGCUGCUCUAGAAAUUUAAGGAAAUUCAU

[0053] 表 1

[0054] 对于各个microRNA，下表2显示microRNA的序列和进入相应前体序列的相应残基（参见表 1）。

[0055]

SEQ ID NO:	名称	序列	来自前体的残基
21	miR-92a-3p	uauugcacuugucccggccugu	48-69
22	miR-92a-1-5p	agguugggaucgguugcaaugcu	11-33
23	miR-92a-2-5p	gggugggguuuguugcauuac	9-30
24	miR-92b-3p	uauugcacucgucccggccucc	61-82
25	miR-92b-5p	agggacgggacgcggugcagug	20-41
26	miR-17-3p	acugcagugaaggcacuuguag	51-72
27	miR-17-5p	caaagugcuuacagugcagguag	14-36
28	miR-503-3p	gggguauuguuucegcugccagg	46-68
29	miR-503-5p	uagcagcgggaacaguucugcag	6-28
30	miR-16-1-3p	ccaguauuaacugugcugcuga	56-77
31	miR-16-2-3p	ccaauauuacugugcugcuuua	53-74
32	miR-16-5p	uagcagcacguaaauauuggcg	14-35 (对于 miR-16-1)或 10-31 (对于 miR-16-2)
33	miR-374a-3p	cuuaucagauuguauuguaauu	42-63
34	miR-374a-5p	uuauaauacaaccugauaagug	12-33
35	miR-374b-3p	cuuagcagguuguauuaucauu	41-62
36	miR-374-5p	auauaauacaaccugcuaagug	11-32
37	miR-374c-3p	cacuuagcagguuguauuauau	39-60
38	miR-374c-5p	auaauacaaccugcuaagugcu	13-34

[0056]

39	miR-24-1-3p	uggcucaguucagcaggaacag	44-65
40	miR-24-1-5p	ugccuacugagcugauaucagu	7-28
41	miR-24-2-3p	uggcucaguucagcaggaacag	50-71
42	miR-24-2-5p	ugccuacugagcugaaacacag	13-34
43	miR-483-3p	ucacuccucuccucccgucuu	48-68
44	miR-483-5p	aagacgggaggaaagaagggag	8-29
45	miR-34a-3p	caaucagcaaguauacugcccu	64-85
46	miR-34a-5p	uggcagugucuuagcugguugu	22-43
47	miR-34b-3p	caaucacuaacuccacugccau	50-71
48	miR-34b-5p	uaggcagugucauuagcugauug	13-35
49	miR-34c-3p	aaucacuaaccacacggccagg	46-67
50	miR-34c-5p	aggcaguguaguuagcugauugc	13-35
51	miR-20a-3p	acugcauuaugagcacuuaaag	44-65
52	miR-20a-5p	uaaagugcuuauagugcagguag	8-30
53	miR-20b-3p	acuguaguaugggcacuuccag	44-65
54	miR-20b-5p	caaagugcucauagugcagguag	6-28
55	miR-15a-3p	caggccauauugugcugccuca	51-72
56	miR-15a-5p	uagcagcacauaaugguuugug	14-35
57	miR-15b-3p	cgaaucauuauuugcugcucua	58-79
58	miR-15b-5p	uagcagcacaucaugguuuaca	20-41

[0057] 表 2

[0058] 除非另有说明，本申请中所指的前体和 microRNA 序列是人序列。然而，在某些情况下，microRNA 人序列与来自其他物种的 microRNA 序列同源。

[0059] 作为一个实例可以提及，人 miR-92a 与来自其他物种的 miR 同源。更具体地，人 miR-92a 与来自 dme(Drosophila melanogaster，黑腹果蝇）、mmu(Mus musculus，小家鼠）、rno(Rattus norvegicus，褐家鼠）、dps(Drosophila pseudoobscura，拟暗果蝇）、aga(Anopheles gambiae，冈比亚按蚊)、dre(Danio rerio，斑马鱼)、mml(Macaca mulatta，猕猴）、xtr(Xenopus tropicalis，热带爪蟾）、ame(Apis mellifera，意大利蜜蜂）、odi(Oikopleura dioica，异体住囊虫）、cin(Ciona intestinalis，玻璃海鞘）、csa(Ciona savignyi，萨氏海鞘）、cfa(Canis familiaris，家犬）和猪或 ssc(Sus scrofa，野猪）的 miR 同源。

[0060] 根据其公知常识，本领域技术人员将容易发现其他人 microRNA 序列和来自其他物种的 microRNA 序列之间的同源性。

[0061] 本文所述的成熟 microRNA 的核酸序列以及它们相应的茎环序列是在在线可检索的 microRNA 序列和注释的数据库 miRBase 中发现的序列。miRBase 序列数据库的进入代表预测的 microRNA 转录物的发夹部分（茎环），以及成熟 microRNA 序列的定位和序列信息。数据库中的 microRNA 茎环序列不是严格的前体 miRNA(pre-miRNA)，而在某些情况下可以包括 pre-imRNA 和来自假定初级转录物的侧翼序列。

[0062] 因此，本发明的目的是提供用于治疗或预防 AMI 后心室重构的组合物，所述组合物包含涉及控制血管生成生理的不同反应的 microRNA 或其前体的抑制剂，或几种 microRNA 或其前体的抑制剂的组合。

[0063] 然而，在大多数发表的研究中，使用静脉途径向动物施用 microRNA 抑制剂。操纵涉及血管基因表达调控的 microRNA 代表缺血性疾病的新治疗靶点。在生物医学方面，对施用尤其是设计成抑制特定 RNA 序列的 RNA 调节器的研究呈指数增长。

[0064] Dimmeler 等已经表明，全身施用特定 microRNA 抑制剂来抑制 miR-92a 改善了左心室功能的收缩性和恢复。因为多顺反子 microRNA17-92a 簇与肿瘤发生相关，也因为 microRNA 细胞型的普遍性，通过静脉重复注射 miR 或抗 miR 引起安全性方面的担忧。

[0065] 由于 microRNA 控制复杂的过程且存在于各种细胞通路这一事实，极有可能它们也引发显著的副作用。在其他之中，目前的一个问题是用 miRNA 抑制剂治疗是非选择性的。

[0066] 此外，由于这些分子的普遍性和低器官特异性，全身施用可能导致在其中这些 miRNA 具有不同细胞特异性功能或它们通常不表达的组织中的运动调控功能。这种错误调控可能会引发副作用。在潜在的风险中，与 microRNA 操纵相关的肿瘤发生仍然是对人体病理学应用这种治疗的主要担忧之一。并且，为了在靶细胞中获得足够持续的浓度，必须以很高的剂量重复注射 microRNA 抑制剂。尽管在控制条件下在小动物上的实验具有较少限制性，换位到人类则意味着生物安全性障碍以及物流和经济障碍。

[0067] 为了解决这些问题以及使这种新的治疗方法能够转移到患者上，考虑产生用于运输 microRNA 抑制剂并将它们直接释放到靶器官的释放介质。合适的介质会将 microRNA 抑制剂直接导向病变组织，从而允许降低剂量以避免重复注射施用，使对其他器官的潜在的不希望的生物作用最小化。

[0068] 因此，目的是通过仅在靶组织中释放 microRNA 抑制剂的手段提高选择性以避免这些治疗的副作用。根据本发明，通过将所述 microRNA 抑制剂微包封成生物可降解且生物相容的微球来解决这个问题。

[0069] 因此，本发明的目的是提供用于治疗或预防 AMI 后心室重构的组合物，所述组合物包含至少一种微包封的 microRNA 的抑制剂，所述 microRNA 属于与调控血管生成相关的 microRNA 家族，所述 microRNA 家族非限制性地包含 miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和 miR-15b），或其前体。

[0070] 通过将 microRNA 抑制剂微包封成微球，促进经腔血管成形术之后的动脉内施用，从而将微球递送并仅保留在受损区域的微血管（也称为毛细血管）中；包封的 microRNA 抑制剂可以以这种方式局部释放。

[0071] 通过 AMI 相关动脉的冠状动脉内注射使微球能够保留在冠状动脉的微循环中，并将 microRNA 抑制剂直接持续释放至靶缺血组织。microRNA 抑制剂诱导新血管生成，这促进受损组织收缩性的功能恢复以及有利的梗死后重构。

[0072] 出乎意料地，本文表明，当微包封成合适的微球时，microRNA 抑制剂，尤其是属于与调控血管生成相关的 microRNA 家族的 microRNA 的抑制剂，所述 microRNA 成功释放至心肌的受损区域从而阻断靶 microRNA 的生物活性。如本文实施例中所示，通过选择性的冠状动脉内途径向经历心肌梗死的个体施用微包封成聚合的生物可降解且生物相容的微球的给定 microRNA 抑制剂，即作为非限制性实例的 miR-92a 抑制剂导致心肌的功能性恢复。

[0073] 因此，保留于微循环中的微球允许 microRNA 抑制剂直接持续释放至靶缺血组织。受损一个月后，在愈合区 microRNA 抑制剂对靶 microRNA 的持续作用（也称为下调）导致明显的血管生长和有害重构的抑制。

[0074] 如本文实施例中所示，已经表明可以通过包封合适的施用于梗死相关动脉的 microRNA 抑制剂来局部和持续抑制属于与调控血管生成相关的 microRNA 家族的 microRNA 来诱导血管生成，从而预防有害心室重构的发生。这代表促进基因调控疗法向患有急性心肌梗死的患者随后安全转移的新递送方法。

[0075] 这些结果清楚表明，如本文所述的包含属于与调控血管生成相关的 microRNA 家族的 microRNA，例如 miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和 miR-15b），或其前体的抑制剂的组合物允许所述抑制剂以治疗有效量以及治疗有效释放速率进行有效的局部释放。

[0076] 在第一个方面，本发明涉及包含有效量的至少一种涉及血管生成的 microRNA 或其前体的抑制剂的组合物，其中将所述抑制剂微包封成聚合的生物可降解且生物相容的微球。

[0077] 在另一个方面，本发明涉及包含有效量的至少一种选自以下的 microRNA 或其前体的抑制剂的组合物：miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和 miR-15b），其中将所述抑制剂微包封成聚合的生物可降解且生物相容的微球。

[0078] 还在本发明的另一个方面，本发明涉及包含有效量的至少一种 miR-92a 或其前体的抑制剂的组合物，其中将所述抑制剂微包封成聚合的生物可降解且生物相容的微球。

[0079] 表述“微球”必须理解为大小为 1 μm 至几百 μm 的球形颗粒。表述“微粒”包括“微球”和“微胶囊”。在本说明书中，使用表述“微球”，但必须理解当本领域技术人员可能且愿意使用“微胶囊”的情况时，使用“微球”和“微胶囊”是等同的。

[0080] 表述“包封”或“微包封”必须理解为包含或包埋于用于保护或用于改性释放的颗粒中。

[0081] 换言之，本发明涉及包含有效量的包封成生物可降解且生物相容的微球的至少

一种涉及血管生成的microRNA抑制剂的药物组合物，所述microRNA抑制剂优选选自包含以下或由以下组成的组：miR-92（包括miR-92a-1、miR-92a-2和miR-92b）、miR-17、miR-503、miR-16（包括miR-16-1和miR-16-2）、miR-374（包括miR-374a、miR-374b和miR-374c）、miR-24（包括miR-24-1和miR-24-2）、miR-483、miR-34（包括miR-34a、miR-34b和miR-34c）、miR-20（包括miR-20a和miR-20b）、miR-15（包括miR-15a和miR-15b）或其前体。

[0082] 换言之，本发明涉及包含有效量的包封成生物可降解且生物相容的微球的至少一种miR-92a或其前体的抑制剂的药物组合物。

[0083] “药物组合物”是指包含药剂的适合向个体施用的物质的混合物。例如，药物组合物可包含miRNA抑制剂和无菌水溶液。

[0084] 在本发明组合物的一个实施方案中，所述涉及血管生成的miRNA由成熟miRNA组成。

[0085] 在本发明组合物的一个实施方案中，所述miRNA由以下成熟miRNA组成：

[0086] a）包含选自SEQ ID NO：21、22或23的序列或与SEQ ID NO：21、22或23之一具有至少90％核苷酸一致性的序列的miR-92a；

[0087] b）包含选自SEQ ID NO：24或25的序列或与SEQ ID NO：24或25之一具有至少90％核苷酸一致性的序列的miR-92b；

[0088] c）包含选自SEQ ID NO：26或27的序列或与SEQ ID NO：26或27之一具有至少90％核苷酸一致性的序列的miR-17；

[0089] d）包含选自SEQ ID NO：28或29的序列或与SEQ ID NO：28或29之一具有至少90％核苷酸一致性的序列的miR-503；

[0090] e）包含选自SEQ ID NO：30、31或32的序列或与SEQ ID NO：30、31或32之一具有至少90％核苷酸一致性的序列的miR-16；

[0091] f）包含选自SEQ ID NO：33、34、35、36、37或38的序列或与SEQ ID NO：33、34、35、36、37或38之一具有至少90％核苷酸一致性的序列的miR-374；

[0092] g）包含选自SEQ ID NO：39、40、41或42的序列或与SEQ ID NO：39、40、41或42之一具有至少90％核苷酸一致性的序列的miR-24；

[0093] h）包含选自SEQ ID NO：43或44的序列或与SEQ ID NO：43或44之一具有至少90％核苷酸一致性的序列的miR-483；

[0094] i）包含选自SEQ ID NO：45、46、47、48、49或50的序列或与SEQ ID NO：45、46、47、48、49或50之一具有至少90％核苷酸一致性的序列的miR-34；

[0095] j）包含选自SEQ ID NO：51、52、53或54的序列或与SEQ ID NO：51、52、53或54之一具有至少90％核苷酸一致性的序列的miR-20；和

[0096] k）包含选自SEQ ID NO：55、56、57或58的序列或与SEQ ID NO：55、56、57或58之一具有至少90％核苷酸一致性的序列的miR-15。

[0097] 在本发明组合物的另一个实施方案中，所述miR-92a由包含SEQ ID NO：21的序列或与SEQ ID NO：21具有至少90％、优选95％的核苷酸一致性的序列的成熟miR-92a组成。

[0098] 在本发明组合物的另一个实施方案中，所述miR-92a由包含SEQ ID NO：22的序列或与SEQ ID NO：22具有至少90％、优选95％的核苷酸一致性的序列的成熟miR-92a组成。

[0099] 在本发明组合物的另一个实施方案中，所述miR-92a由包含SEQ ID NO:23的序列或与SEQ ID NO:23具有至少90％、优选95％的核苷酸一致性的序列的成熟miR-92a组成。

[0100] 在本发明中，两个核酸序列之间的“一致性百分比”是指在最佳比对后获得的两个待比较序列之间相同核苷酸残基的百分比，该百分比纯粹是统计学的，且两个序列之间的差异沿其长度随机分布。两个核酸序列的比较通常通过将它们最佳比对之后比较它们的序列来进行，所述比较能够通过分段或使用“比对窗口”进行。除了手工比较，用于比较的序列的最佳比对还可以通过Smith和Waterman的局部同源性算法(1981)、通过Neddleman和Wunsch的局部同源性算法(1970)、通过Pearson和Lipman的相似性检索方法(1988)或通过使用这些算法的计算机软件(在Wisconsin Genetics Software Package,Genetics Computer Group,575 Science Dr.,Madison中的GAP、BESTFIT、FASTA和TFASTA，或比较软件BLAST NR或BLAST P)来进行。

[0101] 两个核酸序列之间的一致性百分比通过比较两个最佳比对的序列测定，其中与两个序列之间用于最佳比对的参考序列相比，待比较的核酸序列可以具有添加或缺失。一致性百分比通过以下计算:测定两个序列之间，优选两个完整序列之间核苷酸残基相同的位置数，将相同的位置数除以比对窗口中总位置数，将结果乘以100即获得两个序列之间的一致性百分比。

[0102] 如本文所意欲的，与参考序列具有至少90％核苷酸一致性的核苷酸序列涵盖与所述参考序列具有至少91％、92％、93％、94％、95％、96％、97％、98％和99％核苷酸一致性的那些。

[0103] 在本发明组合物的一个实施方案中，所述涉及血管生成的miRNA由microRNA的前体组成。

[0104] 在本发明组合物的一个实施方案中，所述涉及血管生成的microRNA的前体由以下组成:

[0105] a)包含SEQ ID NO:1的序列或与SEQ ID NO:1具有至少90％核苷酸一致性的序列的mir-92a-1;

[0106] b)包含SEQ ID NO:2的序列或与SEQ ID NO:2具有至少90％核苷酸一致性的序列的mir-92a-2;

[0107] c)包含SEQ ID NO:3的序列或与SEQ ID NO:3具有至少90％核苷酸一致性的序列的mir-92b;

[0108] d)包含SEQ ID NO:4的序列或与SEQ ID NO:4具有至少90％核苷酸一致性的序列的mir-17;

[0109] e)包含SEQ ID NO:5的序列或与SEQ ID NO:5具有至少90％核苷酸一致性的序列的mir-503;

[0110] f)包含SEQ ID NO:6的序列或与SEQ ID NO:6具有至少90％核苷酸一致性的序列的mir-16-1;

[0111] g)包含SEQ ID NO:7的序列或与SEQ ID NO:7具有至少90％核苷酸一致性的序列的mir-16-2;

[0112] h)包含SEQ ID NO:8的序列或与SEQ ID NO:8具有至少90％核苷酸一致性的序列的mir-374a;

[0113] i) 包含 SEQ ID NO :9 的序列或与 SEQ ID NO :9 具有至少 90％核苷酸一致性的序列的 mir-374b ；

[0114] j) 包含 SEQ ID NO :10 的序列或与 SEQ ID NO :10 具有至少 90％核苷酸一致性的序列的 mir-374c ；

[0115] k) 包含 SEQ ID NO :11 的序列或与 SEQ ID NO :11 具有至少 90％核苷酸一致性的序列的 mir-24-1 ；

[0116] l) 包含 SEQ ID NO :12 的序列或与 SEQ ID NO :12 具有至少 90％核苷酸一致性的序列的 mir-24-2 ；

[0117] m) 包含 SEQ ID NO :13 的序列或与 SEQ ID NO :13 具有至少 90％核苷酸一致性的序列的 mir-483 ；

[0118] n) 包含 SEQ ID NO :14 的序列或与 SEQ ID NO :14 具有至少 90％核苷酸一致性的序列的 mir-34a ；

[0119] o) 包含 SEQ ID NO :15 的序列或与 SEQ ID NO :15 具有至少 90％核苷酸一致性的序列的 mir-34b ；

[0120] p) 包含 SEQ ID NO :16 的序列或与 SEQ ID NO :16 具有至少 90％核苷酸一致性的序列的 mir-34c ；

[0121] q) 包含 SEQ ID NO :17 的序列或与 SEQ ID NO :17 具有至少 90％核苷酸一致性的序列的 mir-20a ；

[0122] r) 包含 SEQ ID NO :18 的序列或与 SEQ ID NO :18 具有至少 90％核苷酸一致性的序列的 mir-20b ；

[0123] s) 包含 SEQ ID NO :19 的序列或与 SEQ ID NO :19 具有至少 90％核苷酸一致性的序列的 mir-15a ;和

[0124] t) 包含 SEQ ID NO :20 的序列或与 SEQ ID NO :20 具有至少 90％核苷酸一致性的序列的 mir-15b。

[0125] 总体而言，抑制剂是抑制或阻止另一个分子参与反应的分子。

[0126] 如在此所用，术语“miR-X 或 mir-X 的抑制剂”是指减少或降低 miR-X、或 mir-X 或至少一个前体的表达和 / 或活性的任何分子或化合物。因此，这种抑制应当阻止新血管生成抑制，即促进新血管生成从而预防梗死后有害的心肌重构。

[0127] 在本发明的一个实施方案中，所述涉及血管生成的给定 microRNA 抑制剂是长度为 8-49 个核苷酸的具有靶向所述给定 microRNA 的序列的寡核苷酸，所述 microRNA 优选选自 :miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和 miR-15b）或其前体。

[0128] 在另一个实施方案中，所述 miR-92a 抑制剂是长度为 8-49 个核苷酸的具有靶向所述 miR-92a 的序列的寡核苷酸。

[0129] 表述“靶向”是指具有允许与靶核酸杂交以诱导所需效果的核苷酸序列。在某些实施方案中，所需效果是靶核酸的降低和 / 或抑制。

[0130] “杂交”是指通过“核苷酸互补”（即两个核苷酸通过氢键非共价配对的能力）发

生的互补核酸的退火。

[0131] 在一些实施方案中，miRN 抑制剂寡核苷酸的长度为 8-49 个核苷酸。

[0132] 本领域技术人员将理解，这包含长度为 8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48 或 49，或之内任何范围的寡核苷酸。在一些实施方案中，根据本发明的寡核苷酸的长度为 10-20 个核苷酸。本领域技术人员将理解，这包含长度为 10、11、12、13、14、15、16、17、18、19 或 20，或之内任何范围的寡核苷酸。

[0133] 在某些实施方案中，寡核苷酸具有与 miRNA 或其前体互补的序列。

[0134] 在本发明组合物的一个实施方案中，所述寡核苷酸是与涉及血管生成的靶 miRNA 序列至少部分互补的反义寡核苷酸，所述靶 miRNA 优选选自 miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和 miR-15b）或其前体。

[0135] 在本发明组合物的另一个实施方案中，所述寡核苷酸是与 miR-92a 的序列至少部分互补的反义寡核苷酸。

[0136] 表述“反义寡核苷酸”是指具有与特定核苷酸序列（称为有义序列）互补的序列且能够与有义序列杂交的寡核苷酸。

[0137] “互补”是指第一核酸与第二核酸之间的核苷酸配对能力。

[0138] 在某些实施方案中，反义寡核苷酸具有与 microRNA 或其前体互补的核苷酸序列，意味着反义寡核苷酸的序列与 miRNA 或其前体的补体具有至少 60％、65％、70％、75％、80％、85％、90％、95％、97％、98％或 99％的一致性，或两个序列在严格杂交条件下杂交。因此，在一些实施方案中，反义寡核苷酸的核苷酸序列相对于它的靶 microRNA 或其前体序列可以具有一个或多个错配碱基对，并能够与它的靶序列杂交。在某些实施方案中，反义寡核苷酸具有与 microRNA 或其前体完全互补的序列，意味着反义寡核苷酸的核苷酸序列与 miRNA 或其前体的补体具有 100％的一致性。

[0139] 在本发明上下文中，“互补”是指在 8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48 或 49 个核苷酸的区域内，与 miR-92a 的核苷酸序列的补体具有至少 60％、至少 65％、至少 70％、至少 75％、至少 80％、至少 85％、至少 90％、至少 95％、至少 97％、至少 98％、至少 99％或 100％一致性的反义核苷酸或其前体，或是指两个序列在严格杂交条件下杂交。

[0140] “互补性百分比”是指核酸序列中互补核苷酸的数量除以该核酸的长度。在一些实施方案中，寡核苷酸的互补性百分比是指与靶核酸互补的核苷酸的数量除以该寡核苷酸的长度。

[0141] 在一个实施方案中，反义寡核苷酸序列与涉及血管生成的靶 microRNA 的序列“完全互补”，所述靶 microRNA 优选选自 miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和

miR-15b)，更优选为miR-92a或其前体，这意味着反义寡核苷酸的每一个核苷酸均能够与靶microRNA或其前体中各个对应位置的核苷酸配对。

[0142] 在某些实施方案中，根据本发明的反义寡核苷酸具有与以下序列部分或完全互补的序列：

[0143] a)包含选自SEQ ID NO:21、22或23的序列或与SEQ ID NO:21、22或23之一具有至少90％核苷酸一致性的序列的miR-92a；

[0144] b)包含选自SEQ ID NO:24或25的序列或与SEQ ID NO:24或25之一具有至少90％核苷酸一致性的序列的miR-92b；

[0145] c)包含选自SEQ ID NO:26或27的序列或与SEQ ID NO:26或27之一具有至少90％核苷酸一致性的序列的miR-17；

[0146] d)包含选自SEQ ID NO:28或29的序列或与SEQ ID NO:28或29之一具有至少90％核苷酸一致性的序列的miR-503；

[0147] e)包含选自SEQ ID NO:30、31或32的序列或与SEQ ID NO:30、31或32之一具有至少90％核苷酸一致性的序列的miR-16；

[0148] f)包含选自SEQ ID NO:33、34、35、36、37或38的序列或与SEQ ID NO:33、34、35、36、37或38之一具有至少90％核苷酸一致性的序列的miR-374；

[0149] g)包含选自SEQ ID NO:39、40、41或42的序列或与SEQ ID NO:39、40、41或42之一具有至少90％核苷酸一致性的序列的miR-24；

[0150] h)包含选自SEQ ID NO:43或44的序列或与SEQ ID NO:43或44之一具有至少90％核苷酸一致性的序列的miR-483；

[0151] i)包含选自SEQ ID NO:45、46、47、48、49或50的序列或与SEQ ID NO:45、46、47、48、49或50之一具有至少90％核苷酸一致性的序列的miR-34；

[0152] j)包含选自SEQ ID NO:51、52、53或54的序列或与SEQ ID NO:51、52、53或54之一具有至少90％核苷酸一致性的序列的miR-20；和

[0153] k)包含选自SEQ ID NO:55、56、57或58的序列或与SEQ ID NO:55、56、57或58之一具有至少90％核苷酸一致性的序列的miR-15。

[0154] 在某些实施方案中，根据本发明的反义寡核苷酸具有与以下序列部分或完全互补的序列：

[0155] a)包含SEQ ID NO:1的序列或与SEQ ID NO:1具有至少90％核苷酸一致性的序列的mir-92a-1；

[0156] b)包含SEQ ID NO:2的序列或与SEQ ID NO:2具有至少90％核苷酸一致性的序列的mir-92a-2；

[0157] c)包含SEQ ID NO:3的序列或与SEQ ID NO:3具有至少90％核苷酸一致性的序列的mir-92b；

[0158] d)包含SEQ ID NO:4的序列或与SEQ ID NO:4具有至少90％核苷酸一致性的序列的mir-17；

[0159] e)包含SEQ ID NO:5的序列或与SEQ ID NO:5具有至少90％核苷酸一致性的序列的mir-503；

[0160] f)包含SEQ ID NO:6的序列或与SEQ ID NO:6具有至少90％核苷酸一致性的序

列的 mir-16-1 ；

[0161] g) 包含 SEQ ID NO :7 的序列或与 SEQ ID NO :7 具有至少 90％核苷酸一致性的序列的 mir-16-2 ；

[0162] h) 包含 SEQ ID NO :8 的序列或与 SEQ ID NO :8 具有至少 90％核苷酸一致性的序列的 mir-374a ；

[0163] i) 包含 SEQ ID NO :9 的序列或与 SEQ ID NO :9 具有至少 90％核苷酸一致性的序列的 mir-374b ；

[0164] j) 包含 SEQ ID NO :10 的序列或与 SEQ ID NO :10 具有至少 90％核苷酸一致性的序列的 mir-374c ；

[0165] k) 包含 SEQ ID NO :11 的序列或与 SEQ ID NO :11 具有至少 90％核苷酸一致性的序列的 mir-24-1 ；

[0166] l) 包含 SEQ ID NO :12 的序列或与 SEQ ID NO :12 具有至少 90％核苷酸一致性的序列的 mir-24-2 ；

[0167] m) 包含 SEQ ID NO :13 的序列或与 SEQ ID NO :13 具有至少 90％核苷酸一致性的序列的 mir-483 ；

[0168] n) 包含 SEQ ID NO :14 的序列或与 SEQ ID NO :14 具有至少 90％核苷酸一致性的序列的 mir-34a ；

[0169] o) 包含 SEQ ID NO :15 的序列或与 SEQ ID NO :15 具有至少 90％核苷酸一致性的序列的 mir-34b ；

[0170] p) 包含 SEQ ID NO :16 的序列或与 SEQ ID NO :16 具有至少 90％核苷酸一致性的序列的 mir-34c ；

[0171] q) 包含 SEQ ID NO :17 的序列或与 SEQ ID NO :17 具有至少 90％核苷酸一致性的序列的 mir-20a ；

[0172] r) 包含 SEQ ID NO :18 的序列或与 SEQ ID NO :18 具有至少 90％核苷酸一致性的序列的 mir-20b ；

[0173] s) 包含 SEQ ID NO :19 的序列或与 SEQ ID NO :19 具有至少 90％核苷酸一致性的序列的 mir-15a ；和

[0174] t) 包含 SEQ ID NO :20 的序列或与 SEQ ID NO :20 具有至少 90％核苷酸一致性的序列的 mir-15b。

[0175] 在某些实施方案中，根据本发明的反义寡核苷酸具有与 mir-92a-1 的序列 (SEQ ID NO :1) 序列部分互补的序列。

[0176] 在某些实施方案中，根据本发明的反义寡核苷酸具有与 mir-92a-1 的序列 (SEQ ID NO :1) 序列完全互补的序列。

[0177] 在某些实施方案中，根据本发明的反义寡核苷酸具有与 mir-92a-2 的序列 (SEQ ID NO :2) 序列部分互补的序列。

[0178] 在某些实施方案中，根据本发明的反义寡核苷酸具有与 mir-92a-2 的序列 (SEQ ID NO :2) 序列完全互补的序列。

[0179] 在一个实施方案中，反义寡核苷酸包含改性骨架。此类骨架的实例有吗啉代骨架、氨基甲酸骨架、硅氧烷骨架、硫化物、砜和亚砜骨架、formacetyl 和 thioformacetyl 骨架、

methyleneformacetyl 骨架、核糖乙酰 (riboacetyl) 骨架、含烯烃骨架、氨基磺酸酯、磺酸和磺胺骨架、亚甲氨基和亚甲肼基骨架，以及酰胺骨架。

[0180] 吗啉代寡核苷酸具有不带电荷的骨架，其中 DNA 的脱氧核糖被六元环代替，和磷酸二酯键被磷酸二酰胺酯键代替。吗啉代寡核苷酸对酶降解有抗性，且似乎作为反义试剂通过阻止翻译或干扰 pre-mRNA 剪切而不是激活 RNase H 来起作用。

[0181] 改性骨架通常优选提高核酸酶抗性。也可以因为与未改性骨架相比，改性骨架对靶序列具有改变的亲和性而优选改性骨架。未改性骨架可以是 RNA 或 DNA。

[0182] 另一种合适的反义寡核苷酸包含具有改性聚酰胺骨架的肽核酸 (PNA)。基于 PNA 的分子在碱基配对识别方面完全模仿 DNA 分子。PNA 的骨架由通过肽键连接的 7V-(2- 氨乙基)- 甘氨酸单元组成，其中核碱基通过亚甲基羰基键连接至骨架。

[0183] 另一种合适的骨架包含吗啉代核苷酸类似物或等效物，其中核糖或脱氧核糖被六元吗啉环代替。最优选的核苷酸类似物或等效物包含磷酸二酰胺酯吗啉低聚物 (PMO)，其中核糖或脱氧核糖被六元吗啉环替代，且相邻吗啉环之间的磷酸二酯键被非离子的磷酸二酰胺酯键代替。

[0184] 在进一步的实施方案中，本发明的反义寡核苷酸在磷酸二酯键中包含一个非桥氧的取代。这种修饰略微使碱基配对不稳定，但显著增加对核酸酶降解的抗性。

[0185] 本发明另一种合适的反义寡核苷酸包含一个或多个在 2’、3’和 / 或 5’位上单 - 或双取代 (例如 -OH、-F) 的糖部分 ；可能被一个或多个杂原子隔断的取代或未取代的直链或支链的较低级 (CI-CIO) 烷基、烯基、炔基、烷芳基、烯丙基、芳基或芳烷基 ；0-、S- 或 N- 烷基 ；0-、S- 或 N- 烯基 ；0-、S- 或 N- 炔基 ；0-、S- 或 N- 烯丙基 ；0- 烷基 -0- 烷基、- 甲氧基、- 氨基丙氧基 ；- 氨基 xy ；甲氧基乙氧基 ；- 二甲基氨氧基乙氧基和 - 二甲基氨基乙氧基乙氧基。

[0186] 糖部分可以是吡喃糖或其衍生物，或脱氧吡喃糖或其衍生物，优选核糖或其衍生物，或脱氧核糖或其衍生物。这种优选衍生的糖部分包含锁定核酸。

[0187] LNA 是改性 RNA 核苷酸，其中 LNA 核苷酸的核糖部分用额外的连接 2’和 4’碳的桥修饰。这增强了碱基堆叠和预 - 组织，并显著提高了热稳定性。这种桥将核糖“锁定”在通常发现于 A 型 DNA 或 RNA 中的 3’ - 内结构构象中。根据需要，本发明中所用的 LNA 核酸可以与寡核苷酸中的 DNA 或 RNA 碱基混合。

[0188] 根据本发明，所述反义寡核苷酸选自核糖核苷酸、脱氧核糖核苷酸、小 RNA、antagomir、LNA、CDNA、PNA、吗啉代寡核苷酸或它们的组合。

[0189] 在另一个实施方案中，反义寡核苷酸可以由 antagomir 组成。

[0190] 在本发明组合物的一个优选实施方案中，所述寡核苷酸由 antagomir 组成。

[0191] Antagomir 是用来沉默内源 microRNA 的化学工程化寡核苷酸。Antagomir 是与特定靶 microRNA 完美互补但在剪切位点具有错配或某种碱基改性以抑制剪切的小的合成 RNA 或 DNA。通常，antagomir 具有某种改性以使其对降解更加有抗性并促进细胞内在化。Antagomirization(antagomir 抑制 microRNA 活性的过程) 是怎样操作的仍不清楚，但相信它通过不可逆地与 microRNA 结合来抑制。Antagomir 用来连续抑制特定 microRNA 的活性。

[0192] 在本发明的一个实施方案中，所述 antagomir 包含含有至少 8、9、10、11、12、13、

14、15 或 16 个与 microRNA 或其前体互补的连续核苷酸的核苷酸序列，所述 microRNA 的序列选自 SEQ ID NO :1-58。

[0193] 在本发明的一个实施方案中，所述 antagomir 包含含有至少 8、9、10、11、12、13、14、15 或 16 个与 mir-92a 互补的连续核苷酸的核苷酸序列，所述 mir-92a 的序列选自 SEQ ID NO :1、2 或 3。

[0194] 在本发明的一个实施方案中，所述 antagomir 包含含有至少 8、9、10、11、12、13、14、15 或 16 个与 mir-92a 互补的连续核苷酸的核苷酸序列，所述 mir-92a 的序列选自 SEQ ID NO :21、22 或 23。

[0195] 在另一个实施方案中，所述 antagomir 包含含有至少 16 个与 SEQ ID NO :21 的核苷酸序列互补的连续核苷酸的核苷酸序列。

[0196] 在本发明的一个实施方案中，所述 antagomir 具有 DNA 骨架。

[0197] 在所述本发明组合物的实施方案中，所述 antagomir 包含 SEQ ID NO :59 的序列及其除了碱基替换之外的修饰，和由其至少 8 个连续核苷酸的 SEQ ID NO :59 的子序列组成的片段。

[0198] 在本发明的另一个实施方案中，所述 antagomir 具有 RNA 骨架。

[0199] 在所述本发明组合物的实施方案中，所述 antagomir 包含 SEQ ID NO :60 的序列及其除了碱基替换之外的修饰，和由其至少 8 个连续核苷酸的 SEQ ID NO :60 的子序列组成的片段。

[0200] 在一个实施方案中，根据本发明的 antagomir 是由其至少 8 个连续核苷酸的 SEQ ID NO :59 或 60 的子序列组成的片段。

[0201] 在一个实施方案中，根据本发明的 antagomir 是由其至少 9 个连续核苷酸的 SEQ ID NO :59 或 60 的子序列组成的片段。

[0202] 在一个实施方案中，根据本发明的 antagomir 是由其至少 10 个连续核苷酸的 SEQ ID NO :59 或 60 的子序列组成的片段。

[0203] 在一个实施方案中，根据本发明的 antagomir 是由其至少 11 个连续核苷酸的 SEQ ID NO :59 或 60 的子序列组成的片段。

[0204] 在一个实施方案中，根据本发明的 antagomir 是由其至少 12 个连续核苷酸的 SEQ ID NO :59 或 60 的子序列组成的片段。

[0205] 在一个实施方案中，根据本发明的 antagomir 是由其至少 13 个连续核苷酸的 SEQ ID NO :59 或 60 的子序列组成的片段。

[0206] 在一个实施方案中，根据本发明的 antagomir 是由其至少 14 个连续核苷酸的 SEQ ID NO :59 或 60 的子序列组成的片段。

[0207] 在一个实施方案中，根据本发明的 antagomir 是由其至少 15 个连续核苷酸的 SEQ ID NO :59 或 60 的子序列组成的片段。

[0208] 在另一个实施方案中，所述 SEQ ID NO :59 或 60 序列的 antagomir 通过相邻核苷酸之间的磷硫酰键 (phosphotioate bond) 呈现至少 1、2、3、4、5、6 或 7 个改性核苷酸。

[0209] 在另一个实施方案中，所述 antagomir 可以包括 2'-O- 甲基改性的核苷酸、胆固醇基团或任何相似或等效的修饰。

[0210] 通常，如果通过口服或肠胃外施用包含核酸、肽和蛋白质的药物制剂，它在体内被

酶降解，该药物制剂的功效快速消失。已经采用各种尝试来克服这个问题。其中之一是配制长效持续释放注射剂。

[0211] 在实践中，正在对使用支架作为选择性施用 microRNA 的系统进行研究。然而，支架的快速内皮化可能对 microRNA 的持续释放提出问题，尤其是对需要长时间基因调控的生物学过程。此外，已经开发并使用了体内的脂质体和纳米颗粒，但其通过循环系统时具有未能避免的严重副作用风险。此外，没有研究显示不含预先主动脉夹的经皮冠状动脉内施用后的功效和安全性。

[0212] 本发明的另一个方面是使用基于生物可降解且生物相容的微球。

[0213] 根据本发明，基于寡核苷酸与生物相容生物可降解聚合物的微包封，设计并生产了适用于在心脏区域局部释放所述寡核苷酸的释放系统。本发明允许获得基本上载有寡核苷酸的微球，其具有高包封效率，且没有分子改性 / 降解。根据本发明，由于所用的生产条件，尤其是产生的乳剂的特征、聚合物溶液的浓度以及微包封过程中涉及的相体积之间的关系，在没有添加稳定剂或保留物质的情况下保存了分子的纯度和质量。此外，微球具有合适的粒径分布，使其能够在梗死区域的微血管中保留而不造成动脉栓塞，且不会被巨噬细胞的吞噬作用破坏。

[0214] 本发明的目的是提供一种持续释放的微球，其稳定地包封了短链脱氧核糖核酸或短链核糖核酸，并且能够长时间抑制对特定蛋白表达的抑制，尤其是抑制与疾病相关的蛋白。

[0215] 一般来说，“生物相容”是指与活细胞、组织、器官或系统相容，且没有受损、毒性或被免疫系统排斥的风险。生物相容的微球是指该微球以及该微球的任何降解产物对接受者无毒，且对接受者的机体不会产生明显的有害或不良反应，例如在注射位点的免疫反应。

[0216] 一般来说，“生物可降解”是指能够被生物剂的作用分解。

[0217] 如本文所定义，生物可降解的微球是指该微球将在体内降解或损坏以形成更小的化学物质。可以通过例如，酶、化学和 / 或物理过程造成降解。

[0218] 合适的生物相容的、生物可降解的聚合物包括例如，聚（丙交酯）、聚（乙交酯）、聚（丙交酯 - 共 - 乙交酯）、聚（乳酸）、聚（乙醇酸）、聚（乳酸 - 共 - 乙醇酸）、聚己内酯、聚碳酸酯、聚酯、聚酸酐、聚（氨基酸）、聚原酸酯、polyacetyl、聚腈基丙烯酸酯、聚醚酯、聚（对二氧环己酮）、聚（烯烃烷基化物）、聚乙二醇和聚原酸酯的共聚物、生物可降解的聚氨酯、它们的混合物和共聚物。

[0219] 现有技术中已经描述了制备微粒的大量技术。

[0220] 微粒的药物释放谱取决于各种因素，包括所用聚合物的理化性质、聚合物 - 药物 - 赋形剂之间的相互作用和 / 或所得微粒的形态和组成。

[0221] 为了使微球被毛细血管保留，它们必须具有非常特殊的大小分布从而使其一方面被巨噬细胞的吞噬作用，或另一方面动脉栓塞的破坏最小化；使用生物可降解的、生物相容的聚合物的微包封能够从最初几小时直到 2-3 周（AMI 后心室重构发生的主要时期）控制产物的释放。

[0222] 在本发明上下文中，因为优选冠状动脉内途径，这些微球的平均大小需考虑心肌毛细血管的大小；为了在保留在心脏区域，这在 5 到 20，优选 5 到 15 微米之间变化，且没有超过 25 微米的颗粒以防止动脉栓塞。

[0223] 在本发明的一个实施方案中，所述微球的直径不超过 25μm。

[0224] 在一个实施方案中，50 到 100％的微球在 5-25μm 的范围内，没有颗粒超过 25μm。

[0225] 在一个优选的实施方案中，60 到 100％的微球在 5-25μm 的范围内，没有颗粒超过 25μm。

[0226] 在另一个优选的实施方案中，70 到 100％的微球在 5-25μm 的范围内，没有颗粒超过 25μm。

[0227] 在一个更优选的实施方案中，80 到 100％的微球在 5-25μm 的范围内，没有颗粒超过 25μm。

[0228] 根据本发明，微球的平均直径在 5-20μm 的范围内。

[0229] 根据本发明，微球的平均直径在 5-15μm 的范围内。

[0230] 在另一个实施方案中，所述微球的平均直径为 8-11μm。

[0231] 根据本发明的微球将大量所述 microRNA 抑制剂装载入生物可降解且生物相容的聚合物中以在梗死区域持续释放药物。

[0232] microRNA 抑制剂的载量为约 1-20％ (w/w)、优选 1-15％ (w/w)、更优选 1-10，仍然更优选 5-10％。保留药物完整性。

[0233] 在一个实施方案中，根据本发明的微球包含 1％ -15％ w/w 的抑制剂。

[0234] 在另一个实施方案中，根据本发明的微球包含 5％ -15％ w/w 的抑制剂。

[0235] 还在另一个实施方案中，根据本发明的微球包含 1％ -10％，优选 5％ -10％ w/w 的抑制剂。

[0236] 本发明的另一个特征与用于制备微球的聚合物的性质相关。

[0237] 在本发明的一个实施方案中，所述微球用由聚 -d,l- 丙交酯 (PLA) 组成的聚合物制成。在一个实施方案中，所述微球用 PLA 作为唯一的聚合物制成。在一个实施方案中，所述微球用与一种或多种其他生物相容的聚合物共混的 PLA 制成。

[0238] 在本发明的另一个实施方案中，所述微球用由聚 -d,l- 丙交酯 - 共 - 乙交酯 (PLGA) 组成的共聚物制成。在一个实施方案中，所述微球用 PLGA 作为唯一的聚合物制成。在一个实施方案中，所述微球用与一种或多种其他生物相容的聚合物共混的 PLGA 制成。

[0239] 还在在本发明的另一个实施方案中，所述微球用由聚 -d,l- 丙交酯 - 共 - 乙交酯 (PLGA) 和聚 -d,l- 丙交酯 (PLA) 组成的聚合物共混物制成。

[0240] 表述“共混物”必须理解为在将所述混合物溶解于相同有机溶剂之前实现两种或多种聚合物的混合。

[0241] 本领域技术人员将容易理解，与 PLGA 共聚物不同，PLA 聚合物中丙交酯：乙交酯的比例为 100:0 摩尔比。

[0242] 对于 PLGA 共聚物，PLGA 聚合物中丙交酯：乙交酯的比例为 50:50 至 95:5 摩尔比。

[0243] 在一个优选的实施方案中，PLGA 共聚物中丙交酯：乙交酯的比例为 50:50 至 95:10 摩尔比，优选为 50:50 至 80:20 摩尔比。

[0244] 在本发明的一个实施方案中，聚合物的固有粘度为 0.1-0.7dl/g。

[0245] 在一个优选的实施方案中，聚合物的固有粘度为 0.15-0.7dl/g，优选 0.15-0.5dl/g。

[0246] 该方面是令人感兴趣的，因为固有粘度与聚合物分子量相关，因此影响抑制剂从

聚合物微球中释放的速度。

[0247] 因此，本发明的目的还是提供一种用于制备不包括任何助剂且在聚合物组成方面由单一颗粒群组成的微包封的 miR-92a 抑制剂的方法。

[0248] 因此，本发明的另一个方面是用于将 microRNA 抑制剂微包封成聚合物微球的方法，包括：(a) 将 microRNA 抑制剂溶于不包含任何稳定剂的纯化水中；(b) 将聚合物溶于有机溶剂中；(c) 将 (a) 添加至 (b) 以产生第一乳剂；(d) 将步骤 (c) 的乳剂添加至包含表面活性剂和渗透剂的水溶液中以产生第二乳剂；(e) 硬化并收集步骤 (d) 中所得微球；和 (f) 干燥。

[0249] 更具体地，本发明还涉及用于制备如上所述的组合物的方法，其特征在于它包括以下步骤：

[0250] a) 将 microRNA 抑制剂溶于不包含任何稳定剂的纯化水中；

[0251] b) 将聚合物溶于有机溶剂中；

[0252] c) 将 (a) 添加至 (b) 以产生第一乳剂；

[0253] d) 将步骤 (c) 的乳剂添加至包含表面活性剂和渗透剂的水溶液中以产生第二乳剂；

[0254] e) 硬化并收集步骤 (d) 中所得微球；和

[0255] f) 干燥所得微球。

[0256] 本发明的另一个方面是根据本发明的组合物用于治疗心肌梗死的用途。

[0257] 换言之，本发明涉及包含有效量的至少一种 microRNA 或其前体的抑制剂的组合物，其中将所述抑制剂微包封成用于治疗心肌梗死的聚合物生物可降解且生物相容的微球。

[0258] 在一个优选的实施方案中，所述心肌梗死是急性心肌梗死。

[0259] 本发明的还有另一个方面涉及在需要其的受试者中逆转或预防心室重构的方法，包括向所述受试者施用有效量的上述组合物。

[0260] 换言之，本发明涉及包含有效量的至少一种 microRNA 或其前体的抑制剂的组合物，其中将所述抑制剂微包封成在需要其的受试者中逆转或预防心室重构的方法中使用的聚合物生物可降解且生物相容的微球。

[0261] 如已经提及的，根据本发明的组合物适合于通过冠状动脉内途径施用。

[0262] 为了通过冠状动脉内途径施用，微球必须悬浮于合适的介质中，包含或不包含表面活性剂的盐水溶液 (PBS) 或用于静脉施用的另一种合适介质。合适的分散剂包括，例如表面活性剂，如聚山梨酯 80、聚山梨酯 20、聚氧乙烯氢化蓖麻油 60、羧甲基纤维素或聚糖如海藻酸钠；也可能添加等渗剂如氯化钠、甘露醇、山梨糖醇或葡萄糖。考虑到冠状动脉的大小，调节施用介质中微球的浓度以限制血流改变并预防动脉栓塞的风险。该浓度可以在 0.05％ -1％，优选 0.1％ -0.5％之间变化。施用可以通过单次注射或重复注射，接着任选的盐水注射。施用可以在经皮冠状动脉成形术之后，非限制性地施用相同导管进行。

[0263] 本发明方法的特征是，所述施用由通过冠状动脉内途径的施用组成。

[0264] 该方面尤其令人感兴趣，因为它解决了直接静脉施用化合物，例如像 antagomir 一样的寡核苷酸的几种预设的限制。在其他预设的限制中，可以提及 i) 由于 miRNA 的普遍性和低器官特异性的低水平生物安全性，ii) 为了使 microRNA 抑制剂产生其效果的高剂量

和重复注射，和 iii) 计算的静脉剂量的高理论成本。

[0265] 出人意料地，阅读以下实施例后将更加明显，本发明解决了所有这些问题。

[0266] 更具体地，它显示：

[0267] a) 可以在供应病变组织的动脉中选择性施用包封的 antagomir（参见实施例 5）；

[0268] b) 微球保留在动脉中（参见实施例 6）；

[0269] c) 微球的动脉内施用用于肿瘤栓塞，能够永久干扰血流并阻止肿瘤发展，任选地与活性释放物质组合。考虑到这些因素，考虑栓塞风险的存在。为此，以确定微球对心肌不会造成损害或在冠脉流量上产生任何显著改变为目的设计了研究（参见实施例 7）；

[0270] d)microRNA 抑制剂在介质中的良好稳定性以及所述抑制剂从微球的持续释放；这通过它的生物效果同时在施用后长达 10 天抑制 microRNA 来证实（实施例 8）；

[0271] e) 施用具有 miRNA 抑制剂的微球促进受损组织的收缩恢复，并预防有害梗死后重构的发生（参见实施例 9）；

[0272] f) 通过局部施用微球，可以将抑制剂剂量降低至单次注射，这意味着潜在副作用的明显降低和成本的明显减少。

[0273] 根据本发明的用于控制施用、递送和释放 microRNA 抑制剂的合适介质 / 系统的可用性具有以下优势：

[0274] - 提高生物安全性，因为药物在不是治疗靶标的组织和器官中的生物分布是有限的；

[0275] - 避免重复的静脉注射 i) 通过提高患者的支持质量减少住院和门诊就诊，ii) 避免长时间静脉注射药物施用的需要以及由此造成的潜在风险，和 iii) 使静脉注射产物的固有风险（感染、局部反应……）最小化；

[0276] - 减少剂量允许减少剂量依赖性的不良反应；

[0277] - 所需剂量减少使成本降低以及重复注射所需的职员和设备减少。

[0278] 在另一个实施方案中，本发明涉及生物可降解且生物相容的微球群体用于治疗或预防心肌梗死后心室重构的用途，其中所述微球：

[0279] - 平均直径为 5-15 μm；

[0280] - 用聚 -d,l- 丙交酯 - 共 - 乙交酯 (PLGA)、聚 -d,l- 丙交酯 (PLA) 或其共混物制成；

[0281] - 包含 1% -10% w/w 的能够预防心室重构的治疗剂，

[0282] 其中所述治疗剂由涉及血管生成的 microRNA 或其前体的抑制剂组成，优选选自 miR-92（包括 miR-92a-1、miR-92a-2 和 miR-92b）、miR-17、miR-503、miR-16（包括 miR-16-1 和 miR-16-2）、miR-374（包括 miR-374a、miR-374b 和 miR-374c）、miR-24（包括 miR-24-1 和 miR-24-2）、miR-483、miR-34（包括 miR-34a、miR-34b 和 miR-34c）、miR-20（包括 miR-20a 和 miR-20b）、miR-15（包括 miR-15a 和 miR-15b）的 microRNA，更优选 miR-92a，其中所述 microRNA 的抑制剂优选是 antagomir。

[0283] 本发明还涉及试剂盒，包含至少 i) 根据本发明的组合物和 / 或微球；ii) 置放所述组合物的注射器或小瓶或安瓿。

[0284] 在一个实施方案中，本发明的试剂盒进一步包括置于溶剂容器中的溶剂。所述溶剂容器可以是小瓶、安瓿或预充式注射器。

[0285] 微球和溶剂可以置于双隔室预充式注射器中。

[0286] 在一个实施方案中，本发明的试剂盒可以在小瓶中包含微球，在单独小瓶中包含溶剂。

[0287] 在一个实施方案中，本发明的试剂盒可以在小瓶中包含微球，在单独安瓿中包含溶剂。

[0288] 在一个实施方案中，本发明的试剂盒可以在小瓶中包含微球，在预充式注射器中包含溶剂。

[0289] 在一个实施方案中，本发明的试剂盒可以在预充式注射器中包含微球，在单独小瓶中包含溶剂。

[0290] 在一个实施方案中，本发明的试剂盒可以在预充式注射器中包含微球，在单独安瓿中包含溶剂。

[0291] 在一个实施方案中，本发明的试剂盒可以在双隔室注射器中分别包含微球和溶剂。

[0292] 参考以下实施例，本发明将更好被理解。

具体实施方式

[0293] 实施例 1 :制备载有 antagomir-92a 的微球

[0294] 通过 w/o/w 乳剂 / 溶剂蒸发方法制备微球，使用固有粘度为约 0.2dL/g 且包含游离的末端羧基的 50:50PLGA 共聚物。将 3ml 二氯甲烷添加至 0.6gPLGA。将 0.3ml 纯化水中的 Antagomir-92 浓缩溶液 (I-Ssc-miR-92a ;分子量 :5366g/mol(也称为 Da) ;序列 :CCGGGACAAGTGCAAT ;DNA 碱基 :9 ;LNA 碱基 :7 ;生产商 :IDT(Exiqon))(222mg/ml) 添加至 PLGA 有机溶液中，并超声乳化 20s。将这种初级乳剂添加至由 1% (w/v) 聚乙烯醇和 1% (w/v) 氯化钠的水溶液组成的外相，并于约 10300rpm 均质化 60s。将所得的第二乳剂 (w/o/w) 添加至一定体积的纯化水中，通过搅拌蒸发二氯甲烷。将所得微球通过离心收集，用纯化水洗涤两次，然后冷冻干燥。微球的平均直径为 9μm(82％在 5-25μm 之间，0％超过 25μm)，包封效率为 74％。

[0295] 图 1 示出了所得微球的图像。

[0296] 图 2 说明了微球尺寸的分布。

[0297] 实施例 2 :制备载有 RNA 的微球

[0298] 通过 w/o/w 乳剂 / 溶剂蒸发方法制备微球，使用固有粘度为约 0.2dL/g 且包含游离的末端羧基的 50:50PLGA 共聚物。将 3ml 二氯甲烷添加至 0.6gPLGA。将 0.3ml 不含 RNAsa 的纯化水中的 RNA 浓缩溶液 (222mg/ml)(RNA Sigma 5000-10000Da) 添加至 PLGA 有机溶液中，并超声乳化 20s。将这种初级乳剂添加至由 1% (w/v) 聚乙烯醇和 1% (w/v) 氯化钠的不含 RNAsa 的水溶液组成的外相，并于约 10300rpm 均质化 60s。将所得的第二乳剂 (w/o/w) 添加至一定体积的不含 RNAsa 的纯化水中，通过搅拌蒸发二氯甲烷。将所得微球通过离心收集，用不含 RNAsa 的纯化水洗涤两次，然后冷冻干燥。微球的平均直径为 10μm(86％在 5-25μm 之间，0％超过 25μm)，包封效率为 73％。

[0299] 实施例 3 :制备安慰剂微球

[0300] 通过 w/o/w 乳剂 / 溶剂蒸发方法制备微球，使用固有粘度为约 0.2dL/g 且包含游

离的末端羧基的 50:50PLGA 共聚物。将 3ml 二氯甲烷添加至 0.6gPLGA。将 0.3ml 纯化水添加至 PLGA 有机溶液中，并超声乳化 20s。将这种初级乳剂添加至由 1％ (w/v) 聚乙烯醇和 1％ (w/v) 氯化钠的水溶液组成的外相，并于约 10300rpm 均质化 60s。将所得的第二乳剂 (w/o/w) 添加至一定体积的纯化水中，通过搅拌蒸发二氯甲烷。将所得微球通过离心收集，用纯化水洗涤两次，然后冷冻干燥。微球的平均直径为 7μm(84％在 5-25μm 之间，0％超过 25μm)。

[0301] 实施例 4：制备载有白蛋白荧光素异硫氰酸酯的微球

[0302] 通过 w/o/w 乳剂 / 溶剂蒸发方法制备微球，使用固有粘度为约 0.2dL/g 且包含游离的末端羧基的 50:50PLGA 共聚物。将 1ml 二氯甲烷添加至 0.2gPLGA。将 0.1ml 白蛋白荧光素异硫氰酸盐水溶液 (20mg/ml) 添加至 PLGA 有机溶液中，并超声乳化 15s。将这种初级乳剂添加至由 1％ (w/v) 聚乙烯醇和 1％ (w/v) 的水溶液组成的外相，并于约 10300rpm 均质化 60s。将所得的第二乳剂 (w/o/w) 添加至一定体积的纯化水中，通过搅拌蒸发二氯甲烷。将所得微球通过离心收集，用纯化水洗涤两次，然后冷冻干燥。微球的平均直径为 9μm(91％在 5-25μm 之间，0％超过 25μm)。

[0303] 实施例 5：供应靶组织的动脉中选择性施用的研究

[0304] 在大白猪中触发 AMI 之后，通过置于供应梗死区域的对 AMI 负责的动脉的 2.5/12 同轴气球，通过冠状动脉内途径施用 30mg 如实施例 4 所示制备的包含荧光白蛋白的微球。将微球原位悬浮于 10ml 包含 Tween-80 的生理盐水溶液中；连续两次注射 5ml，每次后接着注射 5ml 生理盐水溶液。实验显示可以在供应病变组织的动脉中选择性施用包封的 antagomir。

[0305] 实施例 6：微球保留在病变组织的毛细血管中而不外流至血流的研究

[0306] 通过置于中间前降支的同轴气球通过冠状动脉内途径施用，在猪模型上进行 4 次试验，注射 2 次，每次 5ml 如实施例 4 所示制备的荧光微球。将 4 只动物安乐死，获得毗邻前降支的组织和被其他冠状动脉冲洗的对照组织的心肌样品。通过光学荧光显微镜观察样品，证实存在保留于受损心肌毛细血管中的微球，以及其不存在于对照组织中。

[0307] 为了排除全身性生物分布的可能性，在前述动物的两只中，除缺血和对照心肌组织外，从肺、脾和肝获得 5 个重复样品，用 B 光的光学显微镜观察。仅在前壁心肌壁中检测到荧光。该分析揭示了微球保留在心脏中，避免了 antagomir92a 的全身性释放（减少副作用）。

[0308] 实施例 7：微球保留在病变组织的毛细血管中而不损害靶组织本身的研究

[0309] 进行实验来调查潜在的局部心脏毒性和治疗安全性剂量范围。为了检测局部缺血对心肌的损害，采用了在其检测缺血能力上高度敏感的 2 个配对压电晶体。当心肌组织受缺血影响时，其他组织出现运动障碍并溶胀；这与由其余连续健康组织产生的血压一起使微晶体分离并进一步远离彼此。在进行了开胸手术和心包切除之后的两只猪中插入两对微晶体，一对对照在外侧区，和一对在由前降支（微球通过此施用）供应的前区。对于每对培养基晶体，测量在心脏周期期间的两个点上它们之间的距离：在舒张结束 (EDL) 和收缩结束 (ESL)。EDL 和 ESL 之间的关系用参数 SS(收缩缩短：(EDL－ESL)/EDL) 表示。当左心室收缩完全消失时，EDL ＝ ESL，SS ＝ 0。正常值在 0.2±0.1 的范围。如附图所示，研究剂量诱导了每次注射后持续几秒钟的最小和瞬间振动，这对应于第一次和第二次注射。并且，出乎

意料地，重复冠状功脉内注射根据实施例 4 制备的荧光微球达到研究剂量的 14 倍，没有观察到局部副作用。限制性最大剂量不与不可逆的缺血损害、血液动力学影响 (hemodynamic repercussion) 或心律失常相关。

[0310] 此外，为了检测冠脉流量的变化，将流量传感器置于中间 LAD 来测量冠脉流量。冠状动脉注射后没有观察到冠脉流量的明显变化。

[0311] 结果由其中注射了 120mg 微球的图 3 和其中注射了 240mg 微球的图 4 说明。

[0312] 实施例 8 ：单次冠状动脉内注射具有小剂量 antagomir 的微球的分子效应的研究

[0313] 为了说明小剂量微包封的 antagomir 可以产生分子反应，冠状动脉内施用包封的 antagomir-92a 后测量缺血和对照组织中 miR-92a 的体内表达。在 3 只猪中，将 60mg 根据实施例 1 制备的包含 antagomir-92a 的微球 (0.1mg/Kg) 递送至 LAD。处理后第 1 天、第 3 天和第 10 天将动物安乐死，在 2 个重复梗死和对照样品中，通过总 RNA 分离以及使用特定引物的实时定量 RT-PCR 定量 miR-92a 的表达，以内源 microRNA 作为对照 (miR-123、203 和 126)(参见图 5)。

[0314] 在梗死组织中，miR-92a 的表达与对照组织相比下调了 8 倍，而内源 miR 的表达不受处理影响。抑制从早至第 1 天开始存在，并在第 10 天仍然存在，比对照区的表达水平低 5 倍。

[0315] 在内源 miR 中没有检测到明显调控。这些结果揭示，介质 / 系统为 antagomir-92a 的控制递送和释放提供足够条件，从而用单次冠状动脉内施用产生了 microRNA-92a 的持续抑制。

[0316] 图 6 所示的这些结果也证实了 antagomir 在微球制备过程中没有被降解。

[0317] 实施例 9 ：单次冠状动脉内注射包含低剂量 antagomir 的微球的生物效应的研究

[0318] 为了说明微球输送的 antagomir-92a 的分子效应是否伴随生物效应，用 26 只成年小猪进行了临床前研究。该研究的目的是调查通过选择性冠状动脉内施用包封的 antagomir-92a 来抑制 mir-92a 是否会导致梗死区血管生成的增强，从而预防心室重构的发生。

[0319] 施用 3 种制剂 ：

[0320] - 盐水溶液 (对照制剂)

[0321] - 根据实施例 3 制备的安慰剂微球

[0322] - 根据实施例 1 制备的 antagomir-92a 微球，以 3mg/ 小猪为一次 antagomir 剂量。

[0323] 处理后 4 周，与对照相比，在接受包封的 antagomir-92a 的那些动物中的坏死区中检测到明显更高的血管密度，从而证实了在之前研究中观察到的 antagomir-92s 的促血管生成活性 (161.57±58.71vs 安慰组的 68.49±23.56vs 盐水组的 73.91±24.97，$p = 0.001$)。

[0324] ii) 血管密度 (参见图 7)

[0325] 微血管形成在梗死区以及周边梗死边缘均有增加。那些处理动物中的微血管阻力指数一直显示较低 (200.67±104.46vs 对照的 511.73±202.1，$p = 0.007$)，这与血管密度显著相关 ($R^2$0.41，$p = 0.02$)(参见图 8)。

[0326] 处理组中的基线微循环阻力 (基线 MR) 和实际微循环阻力 (TMR(hyp)) 显著低于对照组 (分别是 7.47±1.33vs 19.62±2.98，$p = 0.005$ 和 5.0±1.15vs 14.49±2.4，p

= 0.006)。基线和实际微循环阻力与血管密度显著相关(分别是 R20.35, p = 0.033 和 R20.31, p = 0.047)(参见图 9)。

[0327] 这些数据表明包封的 antagomir-92a 在体内诱导了持续血管生成。

[0328] 发现生长血管后,因此进一步调查它在 AMI 之后发生的愈合过程中的潜在益处。为了确定包封的 antagomir-92a 对心室重构的作用,比较了处理和未处理组的通过立体磁共振成像 (CMR) 的形态和结构参数,以及通过血管内超声心动图 (IVE) 分析的功能性参数。与处理动物相比,对照在 IVE 中存在明显更高百分比的具有前区和隔顶运动障碍的动物 (p = 0.03)(尤其参见图 10),在离体 CMR 中也具有明显更高的受损心室壁减薄以及左心室中有害重构的形态变化(表 3)。

[0329] 更具体地,图 10 说明了通过血管内超声心动图 (IVE) 分析区域壁运动功能障碍的结果。通过使用 Vivid Q 超声成像器 (GE Healthcare, Belford, UK) 和置于右心室顶端的超声导管 (Siemens) 来进行。

[0330] 本研究的结果表明施用 antagomir-92a 微球与急性心肌梗死后有害重构的统计学上显著的减少有关。

[0331] 表 3 :CMR 中左心室重构的参数

[0332]

	盐水 (N=6)	安慰剂 ME (N=5)	Antagomir-92a ME (N=6)	P
梗死 CMR 片的数量	4.8±0.3	4.8±0.4	5.3±0.2	0.38
$T_{最大梗死壁}$, mm	6.07±0.9	5.61±0.5	9.01±0.6	0.006
$T_{正常后壁}$, mm	13.23±0.5	13.52±1.8	11.82±0.7	0.49
最小减薄百分比, %	54.79±4.9	56.74±4.1	22.71±5.5	0.000
$T_{最小梗死壁}$, mm	3.17±0.4	4.02±0.9	4.35±0.5	0.33
最大减薄百分比, %	76.40±2.18	69.86±4.72	62.54±4.19	0.05
减薄壁的长度, mm	32.2±1.8	31.7±4	20.5±3.6	0.03
D_R/D_N	1.93±0.2	2.02±0.2	1.29±0.1	0.03
D_N, mm	14.88±0.68	13.78±1.59	17.5±1.37	0.12
有害重构% (n)	83.3 (5)	80 (4)	16.7 (1)	0.03

[0333] 包封的 antagomir-92a 阻止在急性心肌梗死 1 个月后的有害的左心室重构。测定各小猪中离体 CMR 的所有梗死片中计算的不同重构参数。示出了四只小猪中代表性的 L2 片(在 L1 顶端)。$T_{最大梗死壁}$ = 平均最大梗死壁厚度,用各片中最大梗死壁厚度的 Σ 除以受影响的片数来计算 ;$T_{正常后壁}$ = 在后乳头肌插入旁边测量的正常后壁的平均厚度,用各受影

响片中后壁厚度的Σ除以受影响的片数来计算；平均最小减薄百分比用[100 - ($T_{最大梗死壁}$ / $T_{正常后壁}$ x100)]来计算；$T_{最小梗死壁}$＝平均最小梗死壁厚度，用各受影响片中最小梗死壁厚度的Σ除以受影响的片数来计算；平均最大减薄百分比用[100 - ($T_{最小梗死壁}$ /$T_{正常后壁}$ x100)]来计算；D_R:梗死壁与对侧正常壁之间的平均最大直径，用各梗死片中梗死壁之间最大直径的Σ除以受影响的片数来计算；D_N:正常壁之间的平均最大直径，与D_R形成直角，与心室腔中心最近，用各梗死片中正常壁之间最大直径的Σ除以受影响的片数来计算；D_R/D_N:平均球形指数，用各梗死片中D_R/D_N的Σ除以受影响的片数来计算。表中数据以平均值 ±s.e.m 表示。

[0334] 代表性 CMR 的结果表明：

[0335] A：小猪 14（诱导 AMI 之后立即死亡）的心脏 NMR 和 IVE：由于紧接着 AMI 之后发生死亡，没有足够的时间触发重构过程。这就是为什么观察到同轴左心室在所有片段中具有相似的尺寸。

[0336] B：AMI 后 20-30 天小猪的心脏 NMR 和 IVE：有害心室重构的证据：AMI 后一个月，在 CMR 上观察到前隔段的极度消瘦，并在 IVE 上观察到动脉瘤形成和运动障碍，这是典型的 AMI 后有害重构。

[0337] C：AMI 后 22-30 天小猪的心脏 NMR 和 IVE：没有心室重构：AMI 后一个月，在 IVE 中观察到前隔区中顶叶区域的轻度减少，没有动脉瘤形成，没有运动障碍。这是 AMI 后有利修复反应的典型情况。

[0338] 实施例 10：包封的 antagomir-92a 诱导血管肿瘤或在短期死亡率中的作用的研究

[0339] 在对所有动物进行的尸检分析中没有观察到血管肿瘤，从而表明在远距离的其他器官中缺少对 microRNA-92a 的异位全身性抑制。研究的死亡率是 23%。没有观察到短期死亡率的差异。只有一只施用包封的 antagomir92a 的小猪死亡 (p ＝ 0.39)。

[0340] 表 4

[0341]

N= 26	盐水 (n=9)	安慰剂 ME (n=9)	Antagomir-92a ME (n=8)
1 个月跟进	6	7	7
死亡	3	2	1

[0342] 实施例 11：对包封的 antagomir-92a 致心率失常谱的研究

[0343] 为了解包封的 antagomir-92a 致心率失常的可能性，通过 Collect 5S 软件 (GE) 记录并分析操作期间所有的心律失常事件。此外，为了解决这个问题，在研究的 26 只小猪中的 10 只中随机植入可插入式循环记录仪以检测潜在心律失常的发作直至死亡（梗死形成以及处理后一个月）。与对照相比，在处理组中没有观察到玛琳心动过速 (maligne tachyarrhythmias) 或心动过缓 (bradiarrhythmias)，表明冠状动脉内包封的 antagomir-92s 没有致心率失常的作用。

[0344] 表 5：研究期间检测到的心律失常

[0345]

	盐水 (n=9)	安慰剂 ME (n=9)	Antagomir-92a ME (n=8)	p
缺血阶段(n= 26)				
没有心率失常, n(%) 心率失常, n(%) • PVC, n • NSVT, n • 室颤, n	2 (22.2) 7 (77.8) 5 0 3	0 (100) 7 1 3	1 (12.5) 7 (87.5) 5 1 3	0.37
再灌注阶段(n=26)				
没有心率失常, n(%) 心率失常, n(%) • 窦性停顿, n • 正常心律,n • IVR, n • PVC, n • NSVT, n	5 (55.6) 4 (44.4) 1 1 0 4 0	3 (33.3) 6 (66.7) 1 0 2 3 0	3 (37.5) 5 (62.5) 0 0 1 3 1	0.6
AMI 后 30 天期间 (n=10)				
植入式循环记录仪	n=4	n=3	n=3	0.88
没有心率失常, n(%) 心率失常, n(%) 窦性心动过速 窦性停顿 PVC 或 PSVC 不可评估	0 (0) 2 (50) 2 0 0 2 (50)	0 (0) 3 (100) 3 1 0 0	0 (0) 3 (100) 3 0 1 0	0.38

[0346] PVC :室性早搏波群 (premature ventricular complexes),NSVT :非持续性室性心动过速,

[0347] IVR :心室自主心律,

[0348] PSVC :室上性早搏波群

[0349] AMI :急性心肌梗死

[0350] 实施例 12 :包封的 antagomir-92a 和未包封的 antagomir-92a 对 miR92a 体外表达的作用的评估

[0351] 12.1 :材料和方法

[0352] a. 细胞

[0353] 使用通过融合原代人脐静脉细胞和肺细胞 A549 的硫鸟嘌呤抗性克隆 (ATCC® CRL-2922™) 建立的人脐静脉细胞系 EA.hy926。

[0354] b. 处理

[0355] 将大约 500000 个 EA.hy926 细胞接种在六孔板上,并于标准条件下 (37℃ ,5% CO2) 在添加有 10%胎牛血清 (FBS) 和 2mM L- 谷氨酰胺 (Sigma,L'Isle d'abeau,France) 的 RPMI1640 中孵育。然后用包含各自 antagomir 及其相应对照 (PBS 或微球) 的新鲜完整 PRMI 培养基替换培养基。用 10 和 150nM 的 antagomir92a(游离的,三批 antagomir92a 微

球)、包封的antagomir17或包封的antagomir20处理EA.hy926细胞。在收集用于提取RNA之前,将细胞进一步孵育24h。提取总RNA,并通过定量RT-PCR定量miRNA的表达。

[0356] c. RNA提取

[0357] 根据生产商的说明(Qiagen,Courtaboeuf,France),分别用Qiagen RNeasy微型版(参考号74106)和RNeasy+通用试剂盒(参考号73404)从EA.hy926细胞和小猪组织中分离miRNA。用NanoDrop ND 1000分光光度计(Labtech International,Paris,France)评估提取的RNA的质量和纯度。

[0358] d. miRNA的逆转录

[0359] 使用TaqMan MicroRNA逆转录试剂盒(Life Technologies,参考号4366596)在包含5ng总RNA和特定miRNA探针的15ml终体积中逆转录miRNA。将样品于16℃孵育30分钟,于42℃孵育30分钟,并通过加热至85℃ 5分钟使逆转录酶失活,然后一直冷却在4℃。

[0360] e. 实时RT-PCR

[0361] 理论基础。从Ct数获得定量值,在该Ct数信号的增加与开始被检测的PCR产物的指数增长相关(根据生产商手册,使用QuantStudio 6 and 7 Flex软件)。为了控制原料量的差异,将数据归一至2个内源对照(miRNA103和miRNA191)的几何平均数,所述对照的表达水平经验上表明不会随处理而变化。随后将靶miRNA的值归一,从而使对照中靶miRNA的值等于1。结果用DDCt计算方法(RQ分析软件,Applied Biosystems®)表示。

[0362] PCR扩增。用QuantStudio™ 6 Flex实时PCR系统和TaqMan探针(Applied Biosystems®)进行所有的PCR反应。热循环条件包括初始变形步骤(95℃,10分钟)和45个循环(95℃,15s和65℃,1分钟)。重复测试样品。

[0363] 12.1:结果

[0364] 10nM游离的和包封的antagomir-92a均抑制了miR-92a的表达,其中下降约90%。

[0365] 150nM游离的和包封的antagomir-92a之后miR-92a的表达不可检测。

[0366] Antagomir-92a处理没有显著减少miR-17或miR-20a的表达。

[0367] 图11总结了这些数据。

[0368] 实施例13:三种包封的antagomir(antagomir17、20a和92a)对其各自miR表达的作用的体外评估

[0369] 13.1制备载有antagomir-17的微球

[0370] 通过乳剂/溶剂蒸发方法使用50:50PLGA共聚物(i.v.0.2dL/g)制备微球。在PLGA有机溶液中乳化antagomir-17(HSA-miR-17-5p;分子量:5305Da;序列:CTGCACTGTAAGCACT;来自Exiqon)溶液。转而将所得乳剂融入分散的水相,并均质化以获得理想的粒径。最后,蒸发溶剂之后,将所得微球冷冻干燥。微球的平均直径为10μm,antagomir-17含量为7.3%。

[0371] 13.2制备载有antagomir-20a的微球

[0372] 通过乳剂/溶剂蒸发方法使用50:50PLGA共聚物(i.v.0.2dL/g)制备微球。在PLGA有机溶液中乳化antagomir-17(HSA-miR-20a;分子量:5289Da;序列:CTGCACTATAAGCACT;来自Exiqon)溶液。转而将所得乳剂融入分散的水相,并均质化以获得理想的粒径。最后,蒸发溶剂之后,将所得微球冷冻干燥。微球的平均直径为10μm,

antagomir-20a 含量为 6.8%。

[0373] 13.3 结果

[0374] 材料和方法与实施例 12 相同。

[0375] -miR-17 被 10nM 包封的 antagomir-17 抑制了 76%。150nM 包封的 antagomir-17 处理完全消除了 miR17 的表达。

[0376] -miR-20a 被 10nM 包封的 antagomir-20a 处理抑制了 7%，被 150nM 包封的 antagomir-20a 处理抑制了 87%。

[0377] 图 12 示出了结果。

[0378] 实施例 14：具有载量、大小和丙交酯 / 乙交酯比例特征的三批 antagomir92a 微球的体外评估

[0379] 14.1 制备具有低 antagomir-92a 载量的微球 (L13250: 聚合物 :RESOMER RG502H)

[0380] 如实施例 1 所述，通过乳剂 / 溶剂蒸发方法使用 50:50PLGA 共聚物 (i.v.0.2dL/g) 制备微球，但采用较低的药物初始量和较高的搅拌速度以制备具有低 antagomir-92a 含量的较小微球。微球的平均直径为 7μm，antagomir-92a 含量为 1.5%。

[0381] 14.2 制备具有高 antagomir-92a 载量的微球 (L13262: 聚合物 :RESOMER RG502H)

[0382] 如实施例 1 所述，通过乳剂 / 溶剂蒸发方法使用 50:50PLGA 共聚物 (i.v.0.2dL/g) 制备微球，但采用较高的药物初始量和较低的搅拌速度以制备具有高 antagomir-92a 含量的较大微球。微球的平均直径为 15.6μm，antagomir-92a 含量为 9.8%。

[0383] 14.3 使用长效聚合物制备载有 antagomir-92a 的微球 (L13230:聚合物 :LACTEL B6006)

[0384] 如实施例 1 所述通过乳剂 / 溶剂蒸发方法制备微球，但采用具有高分子量的 85:15PLGA 共聚物 (i.v.0.64dL/g)，以获得药物缓释。微球的平均直径为 12μm，antagomir-92a 含量为 3.1%。

[0385] 14.4 结果

[0386] 检测具有载量、大小和丙交酯 / 乙交酯比例特征的三批 antagomir92a 微球。

[0387] 根据微球含量，在 2 和 30nM 检测 L13250，在 14 和 210nM 检测 L13262，以及在 4 和 66nM 检测 L13230。

[0388] L13250、L13262 和 L13230 完全消除了 miR92a 的表达。

[0001]

序列表

<110> PIERRE FABRE MEDICAMENT
FUNDACIO HOSPITAL UNIVERSITARI VALL D'HERBRON-INSTITUT DE RECERCA

<120> 包含包封的 antagomir 的组合物

<130> BR96786-PFM

<150> EP 13 305 082.3
<151> 2013-01-24

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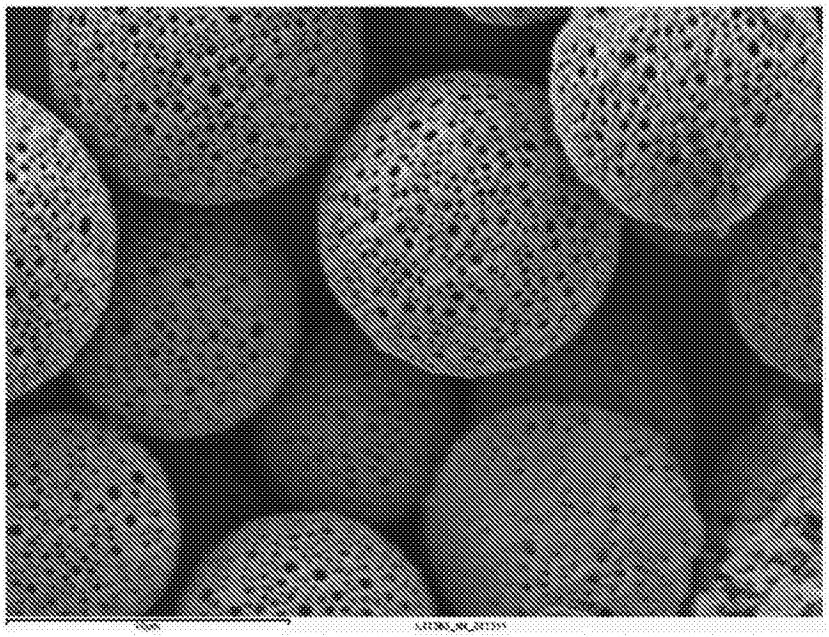
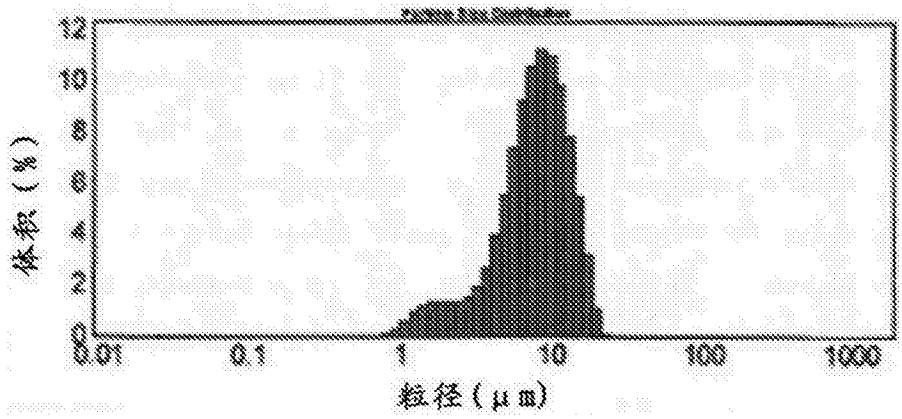


图 1



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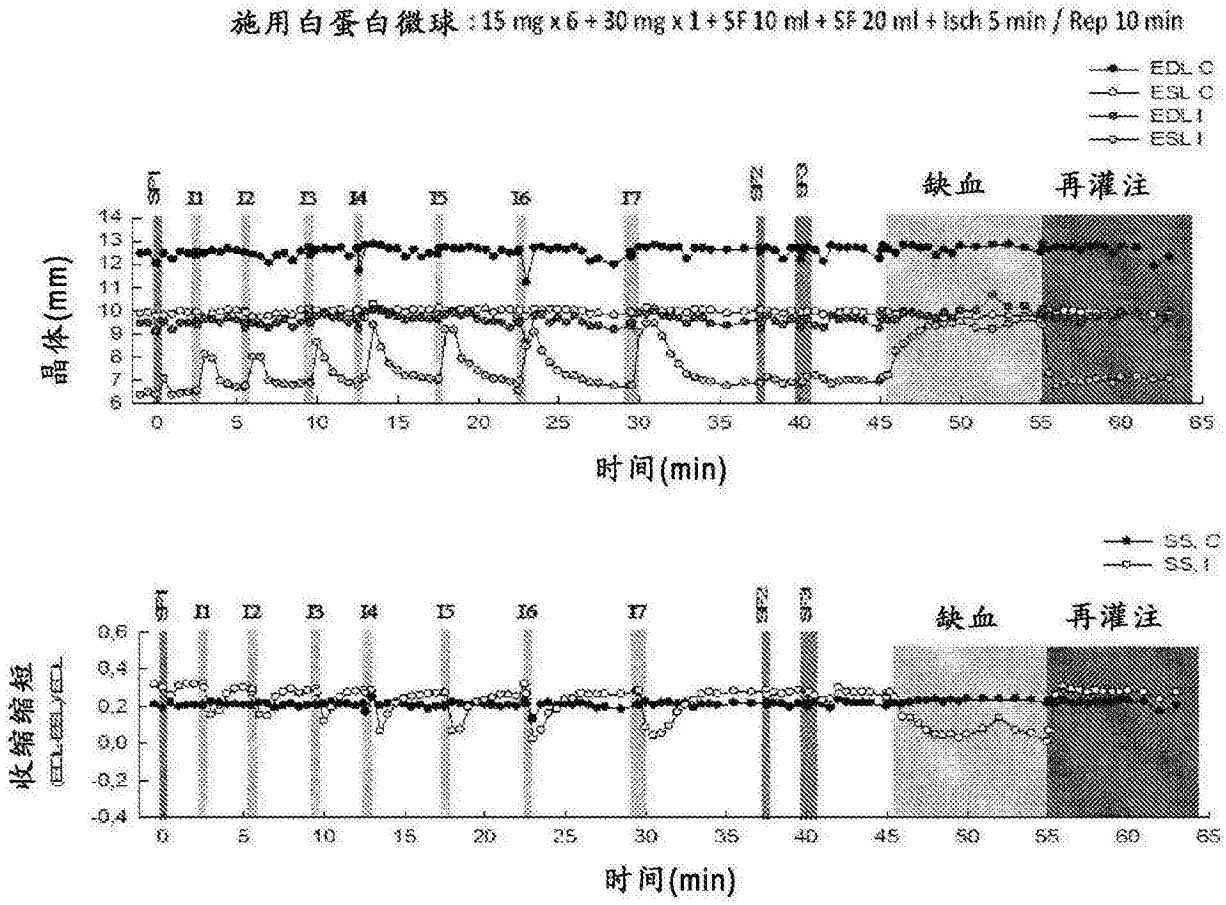
施用白蛋白微球：15 mg x 6 + 30 mg x 1 + SF 10 ml + SF 20 ml + Isch 5 min / Rep 10 min
EDL C
ESL C
EDL I
ESL I
缺血
再灌注
晶体(mm)
时间(min)
SS, C
SS, I
缺血
再灌注
收缩缩短
时间(min)

图 3

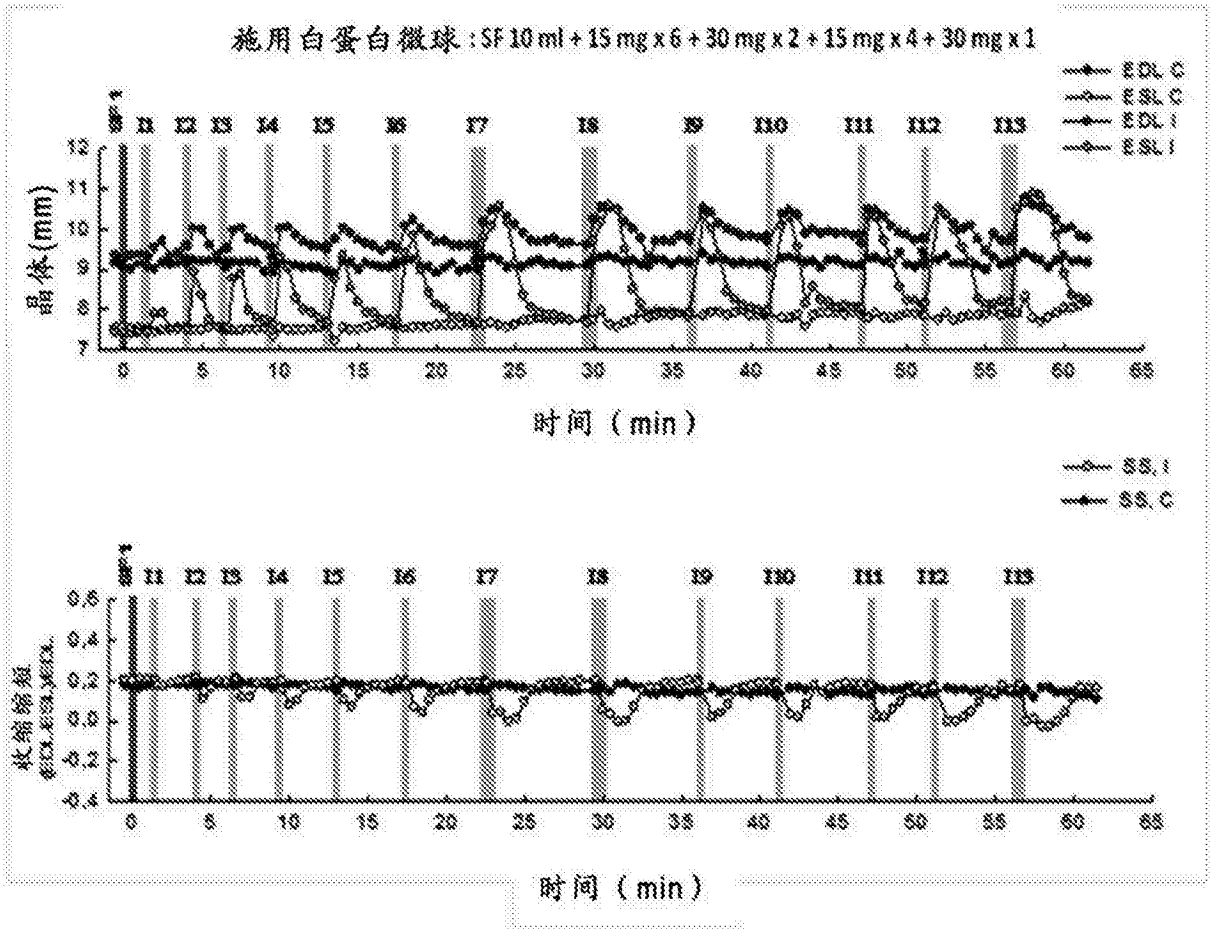
施用白蛋白微球：SF 10 ml + 15 mg x 6 + 30 mg x 2 + 15 mg x 4 + 30 mg x 1
晶体(mm)
时间（min）
时间（min）

图 4

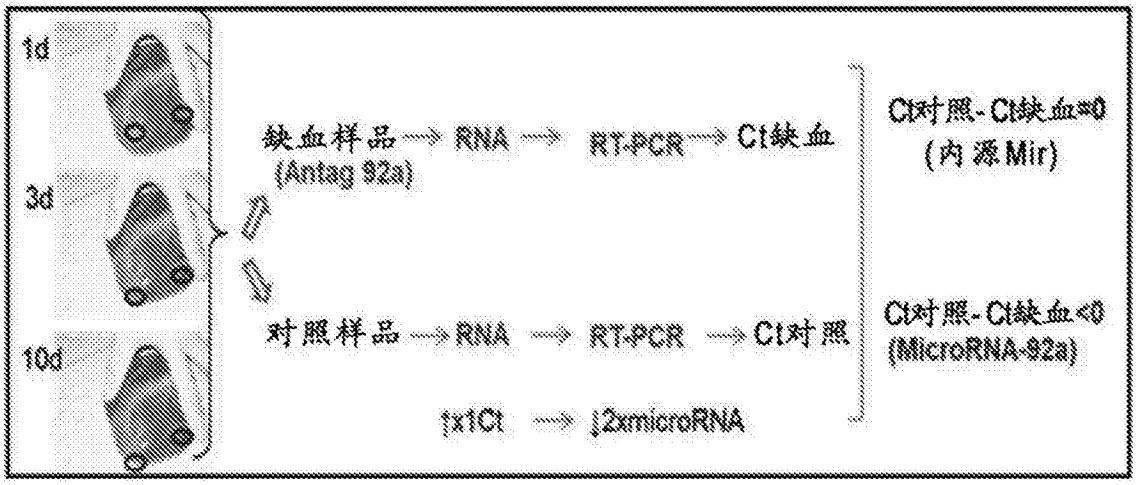
1d
3d
10d
缺血样品 → RNA → RT-PCR → Ct缺血
(Antag 92a)
对照样品 → RNA → RT-PCR → Ct对照
↑x1Ct → ↓2xmicroRNA
Ct对照-Ct缺血=0
(内源Mir)
Ct对照-Ct缺血<0
(MicroRNA-92a)

图 5

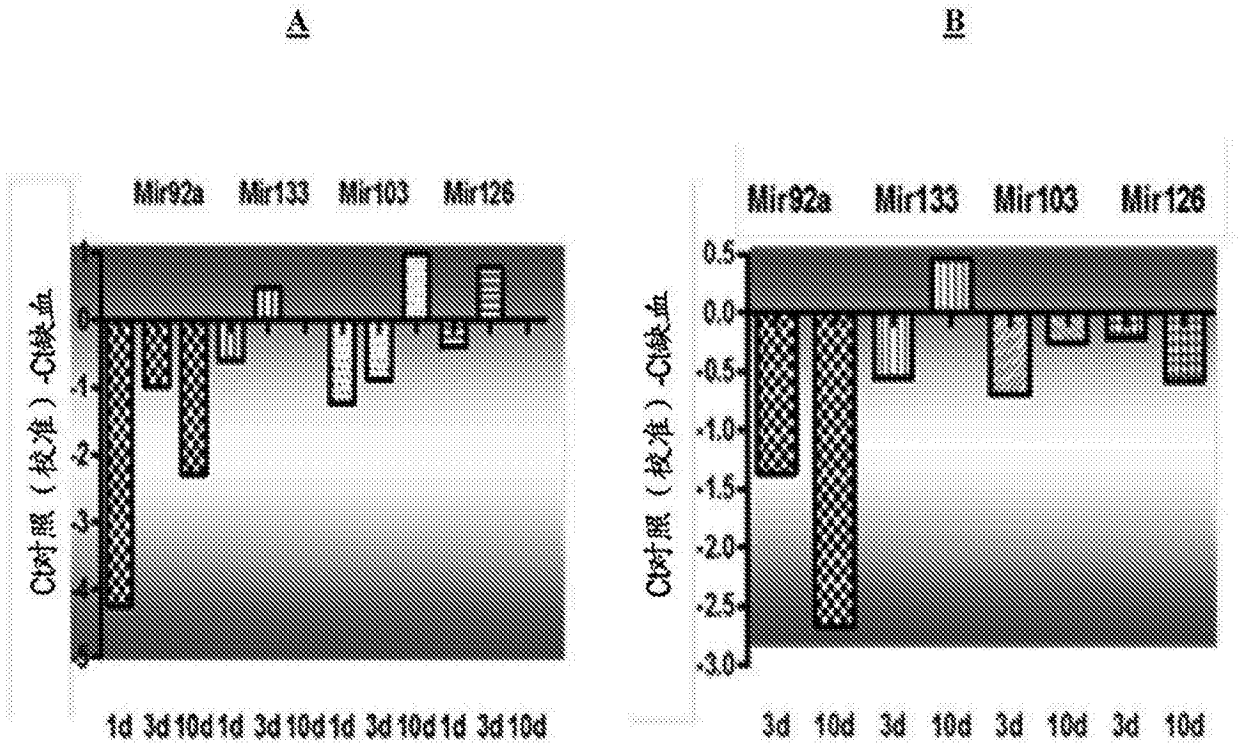
A
B
Mir92a
Mir133
Mir103
Mir126
Ct对照（校准）-Ct缺血
1d 3d 10d 1d 3d 10d 1d 3d 10d 1d 3d 10d
3d 10d 3d 10d 3d 10d 3d 10d

图 6

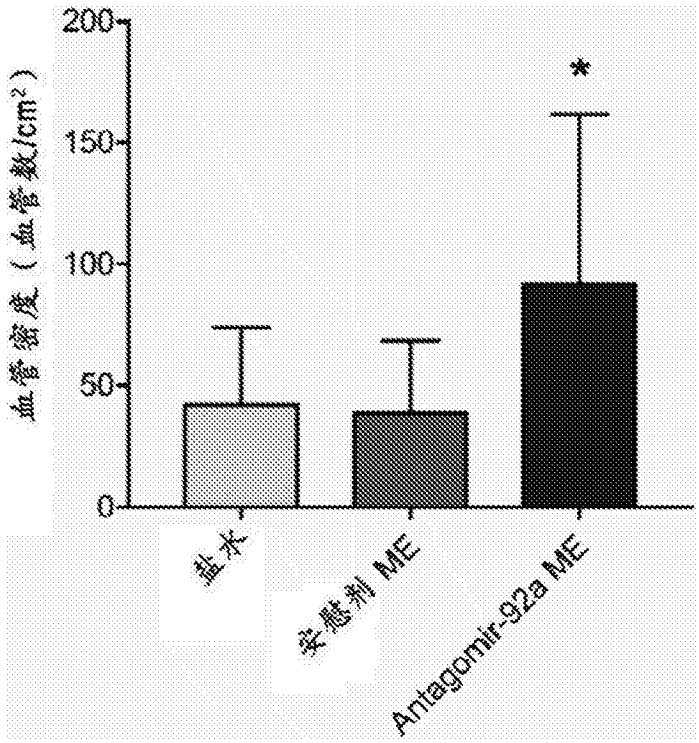
血管密度（血管数/cm²）
200
150
100
50
0
*
盐水
安慰剂 ME
Antagomir-92a ME

图 7

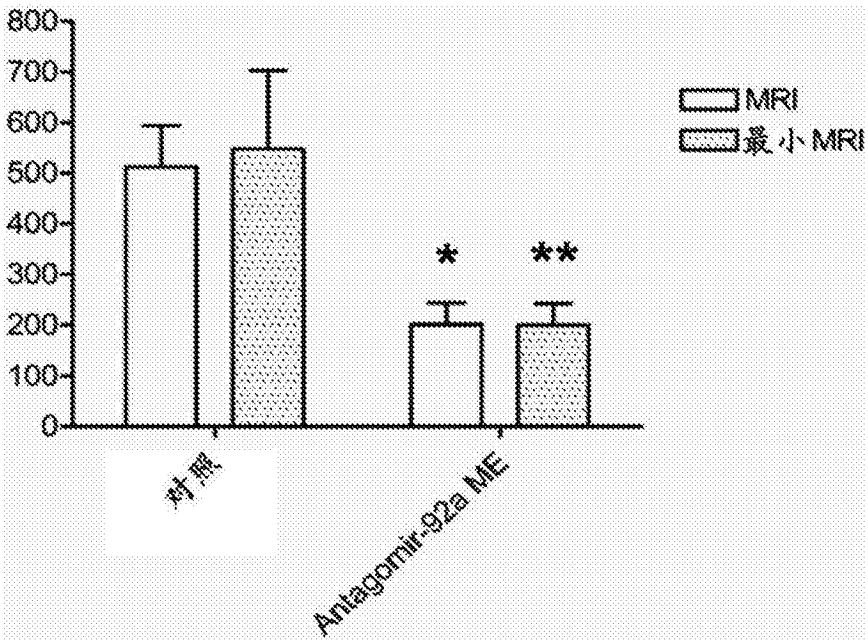
800
700
600
500
400
300
200
100
0
MRI
最小 MRI
*
**
对照
Antagomir-92a ME

图 8

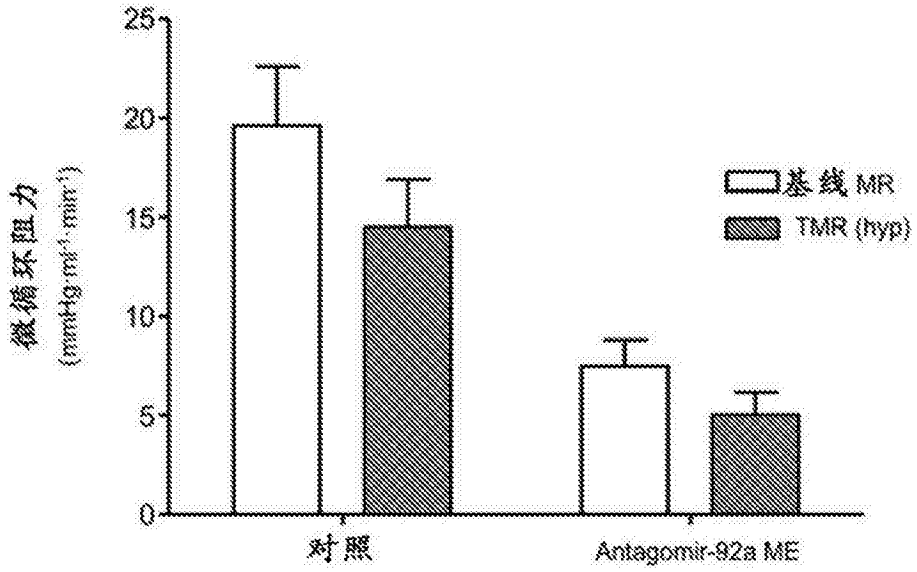
25
20
15
10
5
0
微循环阻力
(mmHg·ml⁻¹·min⁻¹)
基线 MR
TMR (hyp)
对照
Antagomir-92a ME

图 9

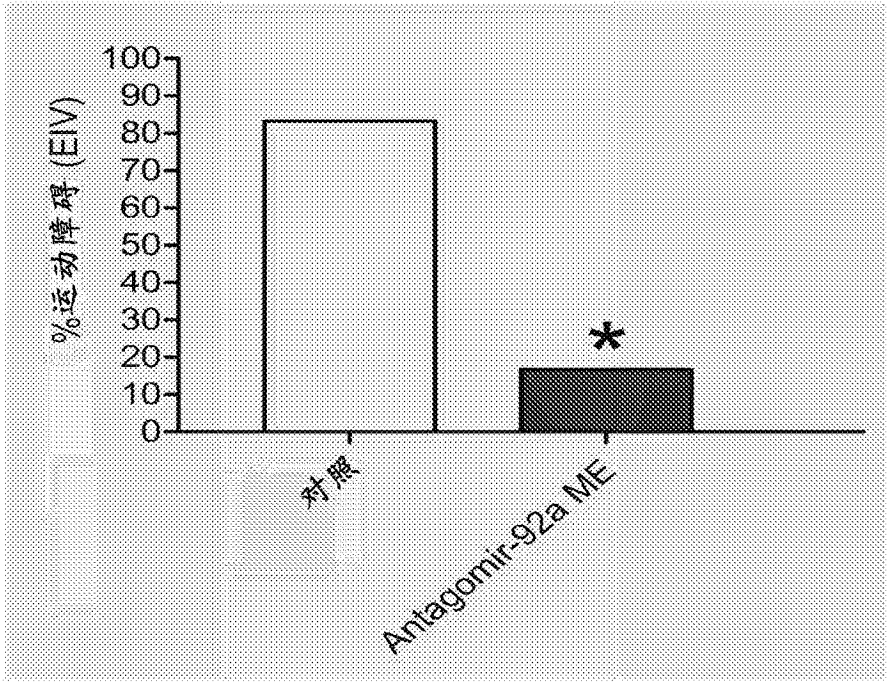
%运动障碍 (EIV)
100
90
80
70
60
50
40
30
20
10
0
*
对照
Antagomir-92a ME

图 10

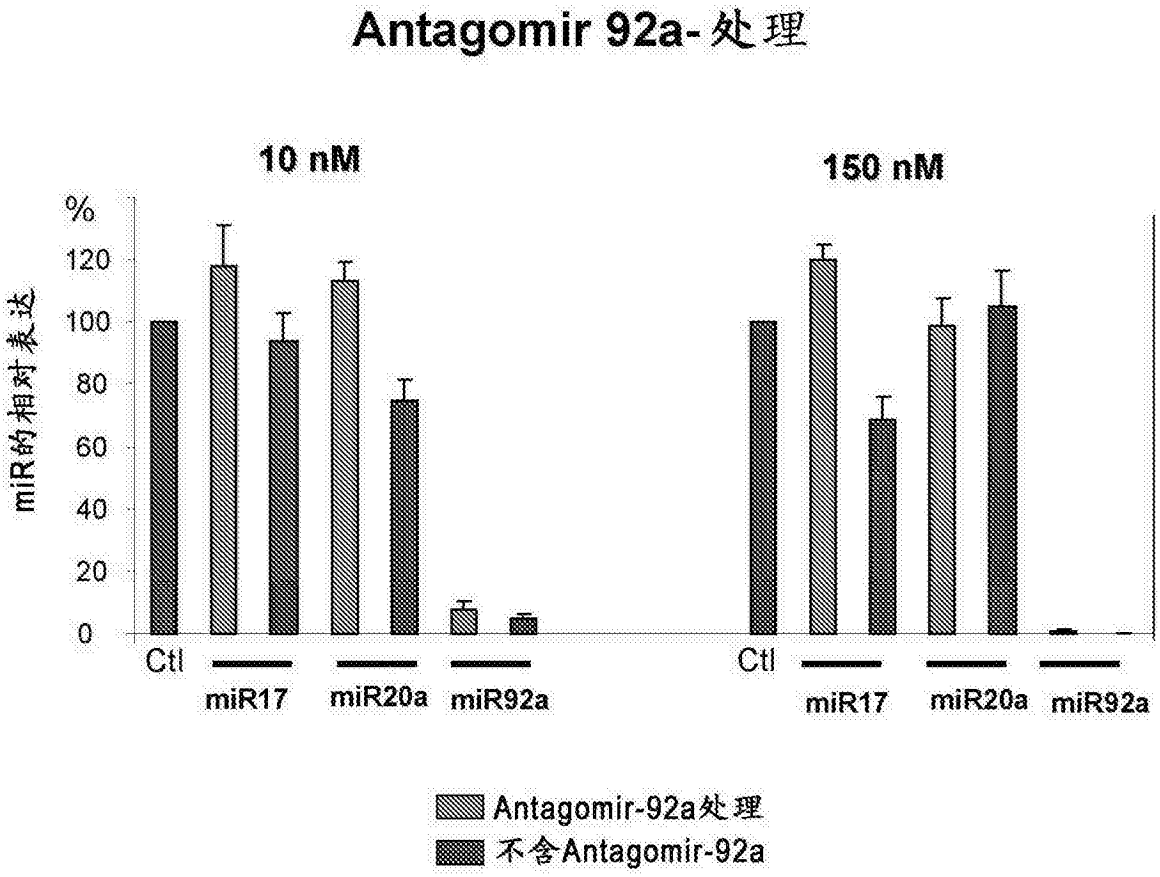
Antagomir 92a-处理
10 nM
150 nM
%
120
100
80
60
40
20
0
miR的相对表达
Ctl
miR17
miR20a
miR92a
Ctl
miR17
miR20a
miR92a
Antagomir-92a处理
不含Antagomir-92a

图 11

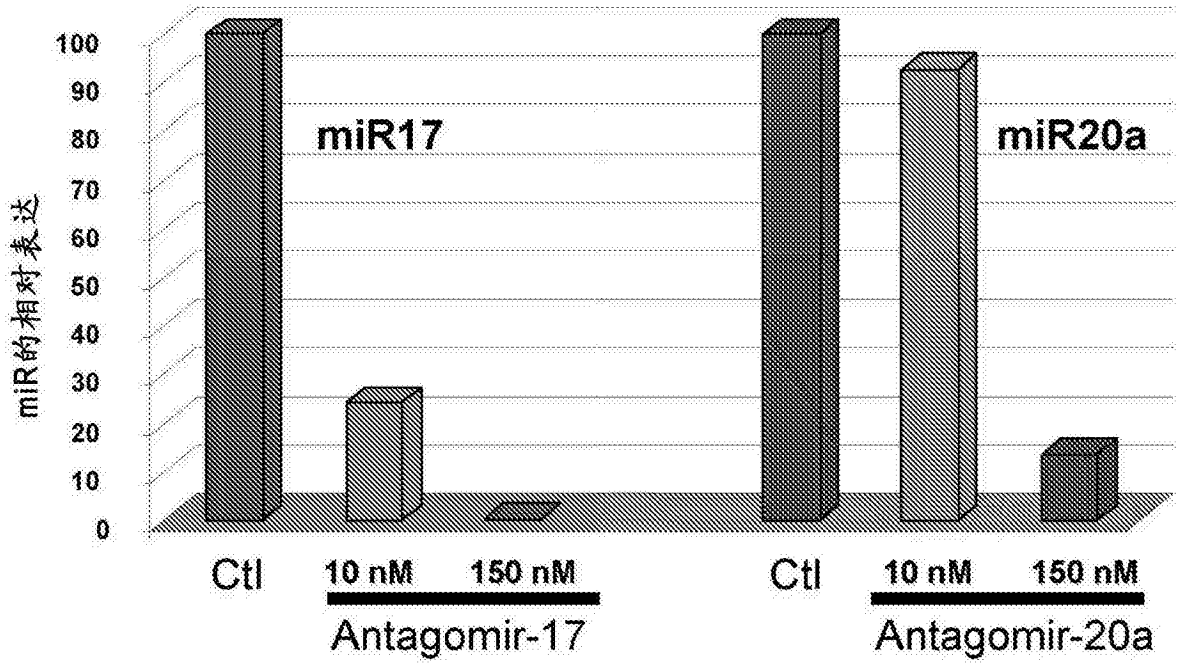
miR的相对表达
100
90
80
70
60
50
40
30
20
10
0
miR17
miR20a
Ctl
10 nM
150 nM
Antagomir-17
Ctl
10 nM
150 nM
Antagomir-20a

图 12