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Development and Characterization of an Active Pharmaceutical Ingredient Using Polymeric Carriers Delivery System

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Abstract

Cardiac diseases are the major cause of mortality, morbidity, and disability. People are dying due to cardiac problems which include atherosclerosis and myocardial infarction. Nanotechnology is the science involving the design, characterization and application of materials in at least one dimension is on nanometer scale. Angiogenesis involves primary vascular plexus formation, which involves differentiation of endothelial cells. Gene therapy makes modification of gene expression for therapeutic gain, where a"normal" gene is inserted into the genome for replacement of an "abnormal" disease-causing gene. A carrier (a vector) is used for delivery of the therapeutic gene to the target cells of patient. Gene delivery involves the ability of DNA for crossing the cell membrane, escaping from endosome, and entering the nucleus. Vascular endothelial growth factor (VEGF) is a potent endothelial cell mitogen in vitro, and a potent permeability mediator and angiogenic growth factor in vivo. Poly (lactide-co-glycolide) (PLGA) is the most used polymer for pharmaceutical controlled release devices. PLGA is biodegradable and biocompatible, hydrolyzing to lactic and glycolic acid monomers. PLGA nanoparticles are potential vector for gene delivery.

Keywords: Cardiac diseases, Angiogenesis, Gene therapy, Vascular Endothelial Growth Factor, Poly-Lactide-co-Glycolide

1. Introduction

Cardiac diseases are the major cause of mortality and morbidity. People are dying of many cardiac problems including atherosclerosis and myocardial infarction.[1] Oral and systemic administration of drugs, does not provide appropriate therapeutic drug levels in the target arteries.[2]

Cardiovascular diseases are the leading causes of death worldwide. Eighty million adults (one in three) in United States are estimated to have one or more types of cardiovascular disease. [3] CAD accounted for approximately 17.8 million deaths in

2017. The number is expected to grow to more than 22.2 million by 2030.[4]

Thus, nanotechnology is an effective treatment modality for achievement of localized and sustained arterial and cardiac drug therapy to prevent cardiovascular diseases. [5]

Gene therapy is used for genetic disorders treatment or prevention by correction of defective genes responsible for development of disease by delivering of repaired genes or the replacement of incorrect ones.[6]

Nanoparticles formulation by PLGA polymer has greater gene transfection demonstration than formulation using PLA polymer in breast cancer this is due to higher DNA release from PLGA nanoparticles.[7]

Angiogenesis is a complex process in which its regulation is done by stimulatory and inhibitory factors and its initiation occurs when there is a predominance of angiogenic factors that favor the growth of new vessel, e.g. VEGF.[8]

Non-viral gene transfer has been used for delivering genes to ischemic tissues for angiogenic therapy. Genes encoding proteins stimulating angiogenic signal transduction are administered by cationic polymers and lipids. [9]

Naked DNA, which is in the plasmid form, is the simplest form of non-viral transfer of the gene into a target cells.[10]

Non-viral gene carriers contain cationic region to condense the anionic therapeutic plasmid, thus protecting the plasmid from degradative nucleases.[11]

Non-viral delivery of genes is safe than its viral delivery counterpart, involving complexing plasmids with cationic polymers or lipids that self-assemble with DNA forming particles able for cells endocytosis.[12]

Polymer-based gene vector are used in intravascular gene therapy utilizes the biocompatible and biodegradable PLGA, which have been approved for use in humans.[13]

The VEGF family has led to discovery of several members: VEGF-A of vascular permeability factor (firstly discovered), B, C, D, E, the family member concerned in angiogenesis is VEGF-A.[14]

PLGA is used polymer for pharmaceutical controlled release devices.[15] The polyester, PLGA is biodegradable and biocompatible, hydrolyzing to monomers of lactic and glycolic acid.[16] PLGA is a drug delivery carrier and FDA-approved as a biodegradable polymer, which has high biocompatiblity. [17]

The present work aimed to detect therapeutic effect of PcDNA3.1-VEGF which was constructed to be injected in rats, targeted to myocytes of infracted rat heart, also to indicate the therapeutic effect of gene transfer complex (Polyplex) formed by coupling of PcDNA3.1-VEGF and PLGA Nanoparticles to be injected in rats.

2. Aim of the work

To detect the therapeutic effect of PLGA – Nanoparticles after gene transfection using PcDNA 3.1-VEGF targeted to myocytes. Also, to evaluate therapeutic effect of PcDNA3.1-VEGF – PLGA – NPs (gene transfer complex, polyplex), the effect of therapeutic material PcDNA 3.1-VEGF and PLGA – NPs – BSA for myocardial infarcted animals treatment.

3. Materials and Methods

3.1Materials:

We used 20 male rats: 4 healthy rats were used as negative control, and 16 animals had induced myocardial infarction with Isoprenaline Hydrochloride.

-Group I (Negative control): Non – injected 4 normal healthy animals.

-Group II (positive control): 4 normal healthy animals were induced myocardial infarction.

-Group III (Treatments): 12 animals, had induced myocardial infarction, and were divided into 3 treatments (4 animals each):

a-Treatment 1: Injected to induce myocardial infarction, then animals were injected with PcDNA 3.1-VEGF.

b-Treatment 2: Injected to induce MI, then animals were injected with PLGA-NPs-BSA.

c-Treatment 3: Injected to induce MI, then animals were injected with PcDNA3.1-VEGF-PLGA-NPs.

A blood sample from rats in each group was taken immediately from normal healthy, infarcted animals after one day, and after three, five, and

seven days of infarction from treatments, then animals were sacrificed and hearts were taken.

3.2. Methods:

3.2.1 The PcDNA3.1- VEGF construct:

a-Many Plasmids used as expression vector can be propagated in Ecoli, PcDNA3.1, was purchased from Invitrogen carrying VEGF.

b-cDNA fragment of human VEGF obtained by reverse transcription-polymerase chain reaction (RT-PCR), using template mRNA, and a set of primers for VEGF

c-PcDNA3.1-VEGF concentration was 372.62ug/ml.

3.2.2Transformation Method for PcDNA3.1 carrying VEGF in E.coli: [18]

3.2.2.1 PcDNA3.1-VEGF description:[19]

Lyophilized 5 μ g of the plasmid carrying VEGF was constructed.

3.2.2.2 Transformation of E.Coli:

a)200 ml competent cells were added to 10 ml PGEM T recombinant for ligation and add 5 ul construct (PcDNA3.1-VEGF) in an epindorf.

b)Keeping on ice for 40 minutes was made.

c)Heat shock at 42 0C for 45 seconds took place.

d)Keeping on ice for 5 minutes was done.

e) 200 ml E.coli recombinant was added to 5 ml LB broth in 50 ml falcon tube.

f)Incubation at 37 ℃ for 2-4 hours , 200 rpm was made

g)Plating on LB/Amp/IPTG/x-gal agar plates took place.

h)Incubation at 37 ℃ overnight was made.

i)Inoculating 1ml E.coli recombinant in 100 ml LB broth and 100 ml Amp was done.

j)Selection of colonies was made.

k)Purifying PcDNA3.1-VEGF from colonies was applied.

3.2.3Preparation and purification of PcDNA3.1 carrying VEGF [20]

3.2.3.1 Principle:

1-Pelleted bacterial cells were resuspended and subjected to SDS/alkaline lysis to liberate the plasmid DNA.

2-Lysate was neutralized for binding of plasmid DNA on membrane in spin column.

3-Cell debris and SDS precipitate were pelleted by centrifugation, and the supernatant containing the plasmid DNA was loaded onto spin column membrane.

4-The adsorbed DNA was washed to remove contaminants, and was then eluted with a small volume of Elution Buffer.

5-The purified plasmid DNA was ready for use in PCR.

3.2.3.2Growth of Bacterial Cultures:

1-Single colony was picked from a freshly streaked selective plate to inoculate 1-5 mL of LB medium supplemented with selection antibiotic and incubated for 12-16 hours at 37° C while shaking at 2000-2500 rpm.

3-Bacterial culture harvested by centrifugation at 8000 rpm 2 min. Decantion of supernatant was done

4-5 mL of bacterial culture was taken.

3.2.3.3Plasmid DNA Purification Protocol:

1-All purification steps were carried out at room temperature.

2-All centrifugations was carried out in micro-centrifuge at 10 000 -14 000 rpm.

3-1-5 mL of E. coli culture in LB media was used for purification.

Plasmid PcDNA3.1-VEGF purification was as follows:

1-The pelleted cells were resuspended in 250 uL of the Resuspension Solution. The cell suspension was transferred to a microcentrifuge tube. Vortexing until no cell clumps remained (RNase was added).

2-250 uL of the Lysis Solution was added and mixed thoroughly.

3-350 uL of the Neutralization Solution was added and mixed thoroughly.

4-Centrifugation for 5 min to pellet cell debris took place.

5-The supernatant was transferred to spin column by pipetting.

6-Centrifugation for 1 min took place. The flow-through was discarded.

7-Spin column was washed by adding 500 uL of Wash Solution I and centrifugated for 30-60 sec. Flow-through was discarded.

8-500 uL of the Wash Solution was added to spin column. Centrifugation for 30-60 seconds took place. Flow-through was discarded.

9-The washing was repeated using 500 uL Wash Solution. Flow-through was discarded and centrifugation 1 min.

10-Spin column was transferred into a fresh 1.5 mL microcentrifuge tube. 50 uL of Elution Buffer was added and elution of the PcDNA3.1-VEGF took place. Purified PcDNA3.1-VEGF was stored at -20° C.

3.2.4Preparation and Purification of RNA:[21]

3.2.4.1. Principle:

Samples were lysed and homogenized in Lysis Buffer, containing guanidine thiocyanate, achaotropic protecting RNA from endogeneous RNases. Lysate was mixed with ethanol and loaded on purification column. Chaotropic salt and ethanol caused RNA to bind to silica membrane. Pure RNA was eluted under low ionic strength.

3.2.4.2Protocol:

1-Blood cells were collected by centrifugation of 0.5 mL of whole blood at 400 ×g for 5 min at 4oC. Blood cells would generate pellet.

2-Resuspension pellet in 600 µ L of Lysis Buffer supplemented with β -mercaptoethanol and vortexing was made.

3-Adding 450 µ L Ethanol (96-100%) and mixing took place.

4-Transferring up to 700 µ L of lysate to Purification Column, centrifugation the column for 1 min at ⩾ 12000 × g and discarding the flow through was made.

5-Repeating this step and discarding collection tube was made.

6-700 µ L Wash Buffer 1 was added to Purification Column and centrifugation 1 min at ⩾ 12000 ×g took place. Flow through was discarded.

7-600 µ L Wash Buffer 2 was added and centrifugation 1 min at ⩾ 12000 × g took place. Flow through was discarded

8-250 µ L Wash Buffer 2 was added and centrifuged 2 min at ⩾12000 ×g.

9-Discarding the collection tube containing flow-through.

10-10-50 µ L Water nuclease-free was added. Centrifugation for 1 min at ⩾ 12000 × g to elute RNA was made.

11- Purified RNA stored at -20° C.

3.2.5Treatment of RNA with DNase I:[22]

3.2.5.1Description:

1-DNase I was an endonuclease that digested single- and double-stranded DNA.

2-Enzyme activity was dependent on Ca2+ and is activated by Mg2+ ions, where DNase I cleaved each strand of dsDNA.

3.2.5.2Removal of genomic DNA from RNA preparations protocol:

1.The following was added to tube:

RNA	1 µL
10X reaction buffer with MgCl2	1 µL
DNase I, RNase-free	1 µL (1U)
DEPC-treated Water	to 10 µL

2. Incubation at 37 ° C for 30 min was made.

3.1 µ L 50 mM EDTA was added and incubated at 65 ° C for 10 min.

4.Prepared RNA was used for reverse transcriptase.

3.2.6Synthesis of cDNA from total RNA:[23]

3.2.6.1Protocol of cDNA synthesis:

3.2.6.1.1Preparation of 2X RT master mix:

1) Kit components were thawn in ice.

2)Volume of components was as follows:

3)2 X RT master mix was placed on ice and mixed.

Table 1: cDNA Reverse Transcription Kit Components

component	Volume reaction (ul) With RNase Inhibitor
10X RT Buffer	2.0
25X dNTP Mix(100mM)	0.8
10X RT Random primers	2.0
Reverse transcriptase	1.0
RNase Inhibitor	1.0
Nuclease-free H_2O	3.2
Total per Reaction	10.0

3.2.6.1.2Preparation the cDNA Reverse Transcription Reactions:

a)10 ul of 2 X RT master mix was pipette into tubes.

b)10 ul of RNA sample was pipette into each tube. The tubes were sealed.

c)Centrifugation was done.

d)The tubes were placed on ice then loaded on thermal cycler.

3.2.6.1.3Performing reverse transcription:

1)The thermal cycler was programmed:

Table 2:Conditions and Steps for Thermal Cycler

Condition	Step1	Step 2	Step 3	Step 4
Temperature (^{0}C)	25	37	85	4
Time	10 min	120 min	5 min	∞

2)The reaction volume was set to 20 uL.

3)The reactions were loaded into thermal cycler.

4)The reverse transcription run was started.

5)cDNA was stored at 2 to 6 ℃.

3.2.7Quantization of mRNA using SYBR Green qPCR:[24]

Rotor Gene Q-QIAGEN was used.

-Protocol:

1-Gently vortexing and centrifugation were made.

Reaction master mix was prepared:

Table 3: Components of SYBR Green Kit

Maxima SYBR Green qPCR Master Mix (2X)	12.5 ul
Forward Primer	0.3 uM
Reverse Primer	0.3 uM
Template DNA	＞500ng
Water nuclease free	To 25 ul
Total volume	25 ul

3-Master mix was mixed thoroughly and dispersed into PCR tubes.

4-The template DNA was added to PCR tubes containing master mix.

5-Reaction was mixed and Centrifugated.

6-Thermal cycler was programmed:

Table 4: Steps, Temperature, Time and Number of Cycles for qrt-PCR

Step	Temperature ^{0}C	Time	Number of cycles
Initial Denaturation	95	10 min	1
Denaturation	94	30 sec	50
Annealing	57	30 sec	
Extension	72	30 sec	

3.2.8Preparation of PLGA Nanoparticles:[25]

3.2.8.1Materials needed for PLGA nanoparticles preparation:

1-PLGA [Sigma- Aldrich].
2-Bovine serum albumin (BSA).
3-Dichloromethane (DCM).
4-Poly-vinyl alcohol (PVA) [Sigma-Aldrich].
5-Milli-Q water.

3.2.8.2Steps for Preparation of PLGA Nanoparticles:

a)PLGA was dissolved in organic phase (4ml), sonicated with addition of IAP (Internal Aqueous Phase), to make primary emulsion.

b)For formation of secondary emulsion 16 ml of EAP (External Aqueous Phase), was added drop wise into primary emulsion during sonication.

c)Secondary emulsion was kept in magnetic stirrer overnight to evaporate excess DCM.

d)PLGA Nanoparticles were separated through centrifugation at 15000 rpm for 20 mins.

e)Separated particles were washed twice with MQ water then lyophilized and stored in -20℃.

f)All of the above steps were done for preparation of PLGA-NPs- BSA.

g)Same methodology was done for PcDNA3.1-VEGF, but 10ml of EAP was instead of 16ml.

3.2.8.3Preparation of PLGA-NPS by W/O/W technique:

The following table shows reagents used for preparation of nanoparticles and gene transfer complex.

Table 5: Various PLGA nanoparticles Preparations reagents

Preparations	IAP				EAP	
	PcDNA3.1-VEGF	PLGA	BSA	DCM	MQ – water	PVA
PLGA-NPs alone	Non	0.2 g	Non	4 ml	16 ml	0.16 g
PLGA-NPs - BSA	Non	0.2 g	800ul	4ml	16 ml	0.16 g
PcDNA3.1-VEGF- PLGA-NPs	250 ul	0.125 g	Non	2.5 ml	10 ml	0.19 g

3.2.8.4Characterization of PLGA nanoparticles:

3.2.8.4.1Morphology:[26]

This was done using Jsm 1400 -+ Jeol Japan TEM Transmission Electron Microscope.

3.2.8.4.2Particle size analysis:[26]

Analyzing of samples was done using Zetasizer Nano ZS Size Analyzer (Malvern, UK).

3.2.8.4.3Zeta potential:[27]

This was determined using Zetasizer Nano ZS size analyzer, (Malvern, UK).

3.2.9Measuring Cardiac Enzymes of injected and non-injected animals (Cardiac profile):

3.2.9.1Creatine Kinase (CK):[28]

3.2.9.1.1Principle:

CK catalyzed phosphorylation of ADP, in presence of creatine phosphate to form ATP and creatine. Catalytic concentration was determined

from the rate of NADPH formation; measured at 340 nm.

Creatine phosphate + ADP $\xrightarrow{CK}$ Creatine + ATP

ATP + Glucose $\xrightarrow{H}$ ADP + Glucose-6-phosphate

Glucose-6- phosphate + $NADP^+$ $\xrightarrow{G6PDH}$ 6-phosphogluconate +NADPH +H^+

3.2.9.1.2Procedure:

1-One milliliters working solution and 50ul serum were pipette into cuvette.

2-Mixing and incubation 3 minutes took place.

2-Absorbance (A) of sample was read.

3-Average absorbance differences were calculated.

4- Δ A/min X 3333 = U/L CK was performed.

3.2.9.2Creatine Kinase MB (CK-MB):[29]

3.2.9.2.1Principle:

Specific antibody inhibited M subunits of CK-MM and thus allowed determination of B subunit of CK-MB, catalytic concentration was determined from rate of NADPH formation, measured at 340 nm.

Creatine phosphate + ADP $\xrightarrow{CK}$ Creatine + ATP

ATP + Glucose $\xrightarrow{HK}$ ADP + Glucose-6- phosphate

Glucose-6- phosphate + $NADP^+$ $\xrightarrow{G6PDH}$ 6-phosphogluconate +NADPH +H^+

3.2.9.2.2Procedure:

1-One milliliter working solution and 50ul serum was pipette into cuvette.

2-Mixing and incubation for 3 minutes took place.

3-Initial absorbance (A) was read.

4-Average absorbance differences were calculated.

5- Δ A/min X 6666 = U/L CK-MB was performed.

3.2.9.3Lactate dehydrogenase (LDH):[30]

3.2.9.3.1Principle:

LDH catalyzed reaction between pyruvate and NADH to produce NAD^+ and L-Lactate:

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L-Lactate + NAD^+

The initial rate of NADH oxidation was directly proportional to catalytic LDH activity and was determined by measuring decrease in absorbance at 340 nm.

3.2.9.3.2Procedure:

1-one milliliter of working solution was added to 20 ul specimen.

2-Mixing and measuring of absorbance after 30 seconds took place.

3-Mean absorbance was performed.

4-LDH activity was calculated using U/L = 8095 x Δ A at 340 nm/min.

3.2.10Investigation of the myocardium:

1.The cardiac tissue was fixed at 10% ne formaldehyde.

2.Then dehydration in ascending grades of alcohol (70-100%) was done.

3.Clearing in xylene took place.

4.Impregnation in molten paraffin at 60 OC was made.

5.The blocks were cut into 5 µm thick sections with microtome.

6.The paraffin sections were depraffinized with xylene then rehydrated in alcohol (70-100%).

7.The slides were rinsed in haematoxylin stain, and then rinsed in eosin stain.

8.Then slides were dehydrated in the ascending series of alcohol and cleaned with xylene.

The slides were mounted by DPX, Covered and Photographed using Olympus light microscope

3.2.11Analyzing the QRT-PCR Data using Comparative CT Method (Δ Δ CT):

Mean CT values were used in Δ Δ CT calculations. Fold change expression of VEGF after treatment, was calculated:

3.2.11.1Calculations of the Δ CT:

Δ CT = CT target gene (VEGF) -CT reference gene (B-actin)

3.2.11.2Calculations of the Δ Δ CT:

Δ Δ CT = Δ CT test sample - Δ CT calibrator sample, by subtracting the Δ CT of untreated from the Δ CT of Treated. Calculation of fold expression was: 2 - Δ Δ CT.

3.2.12Statistical analysis of the data:

Using IBM SPSS software package version 20, ANOVA was performed.

4. Results

4.1Agarose gel electrophoresis for PcDNA3.1-VEGF:

Isolated plasmid DNA was run on 1% agarose gel electrophoresis to check integrity of plasmid. Isolated plasmid was supercoiled in conformation. Plasmid had Ampicillin resistance gene to serve as selection marker. Concentration and purity of plasmid DNA were determined by Nanodrop device (Jenway). Three bands of PCDNA3.1-VEGF appeared in the Agarose gel (marker 1K), highest band is for circular plasmid, middle is for linear plasmid lowest is for super coiled plasmid.

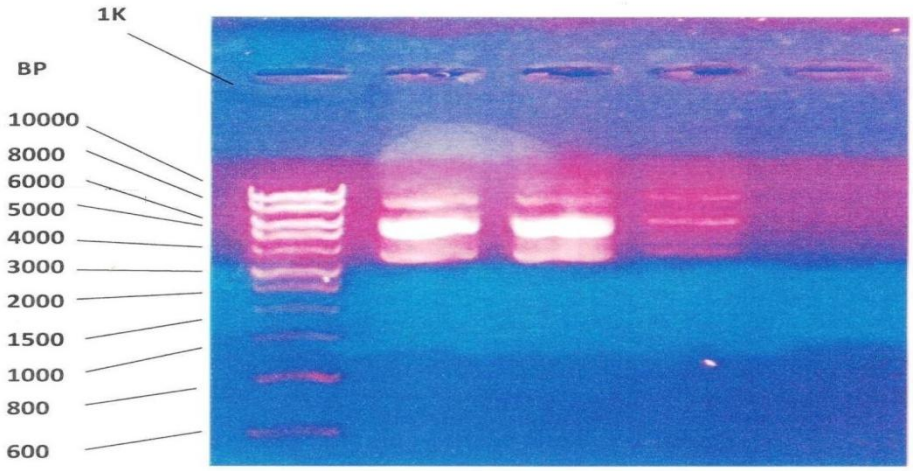

Figure 1: Agarose gel electrophoresis of PcDNA3.1–VEGF

Table 6: Concentration and purity of PcDNA3.1 -VEGF

Concentration of PcDNA3.1-VEGF ug/ml	Purity (260/280)	Purity (260/230)
372.62	1.776	0.916

4.2 Characterization of PLGA-NPs:

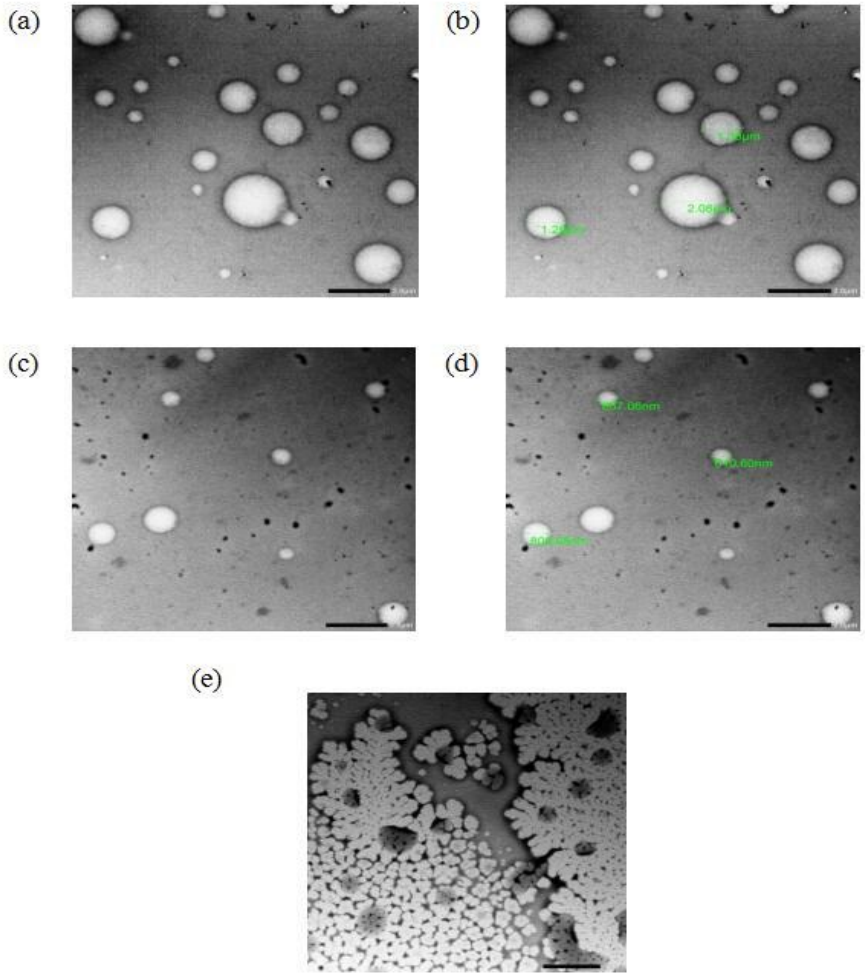

Figure 2: (a) TEM image for PLGA-NPs alone, (b) Particle size for PLGA-NPs alone, (Mean particle size is 1.58 um), (c) TEM image for PLGA-NPs – BSA, (d) Particle size of PLGA-NPs-BSA, (mean particle size is 691.4 nm), (e) TEM image of gene transfer complex (polyplex)

4.2.1 Zeta-sizer and Zeta potential:

Zeta potential measures degree of repulsion between adjacent, charged particles in dispersion, a high zeta potential indicates stability.

Table 7: Size and % intensity values of different samples

Sample	Peaks	size	% intensity	St. Dev.	Z-average	Intercept	PDI
PLGA-NPs alone	Peak 1	697.6	61.4	233.1	500.8	0.970	0.890
	Peak 2	131.0	25.4	39.39			
	Peak 3	24.63	7.4	4.296			
PLGA-NPs-BSA	Peak 1	863.1	58.9	149.8	2494	0.969	1.000
	Peak 2	414.8	29.1	39.70			
	Peak 3	33.86	7.6	3.861			
PcDNA3.1-VEGF–PLGA-NPs	Peak 1	694.9	89.1	126.0	1422	0.877	0.946
	Peak 2	78.01	10.9	17.72			
	Peak 3	0.000	0.0	0.000			

Table 8: Zeta potential and Zeta deviation values of different samples

Sample	Peaks	Mean	Area (%)	St. Dev.	Zeta Potential	Zeta Deviation(mv)	Conductivity (mS/cm)
PLGA-NPs alone	Peak 1	-3.25	53.1	3.96	-11.9	10.6	0.0354
	Peak 2	-21.7	46.1	5.84			
	Peak 3	0.00	0.0	0.00			
PLGA-NPs-BSA	Peak 1	2.85	100	4.42	2.85	4.42	0.0645
	Peak 2	0.00	0.0	0.00			
	Peak 3	0.00	0.0	0.00			
PcDNA3.1-VEGF–PLGA-NPs	Peak 1	-14.3	100	6.86	-14.3	6.86	0.0243
	Peak 2	0.00	0.0	0.00			
	Peak 3	0.00	0.0	0.00			

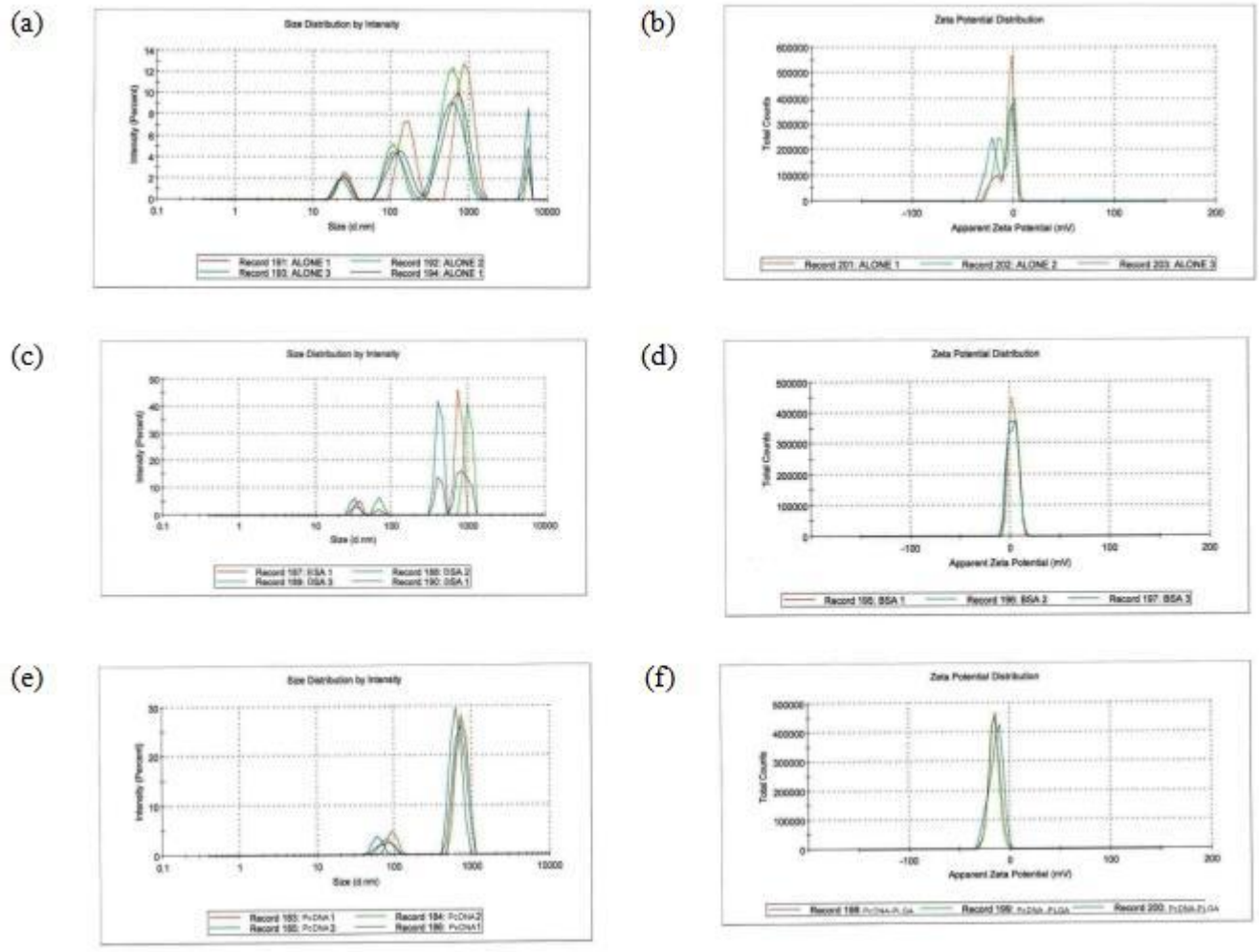

Figure 3: (a) Size Distribution for PLGA-NPs, (b) Zeta potential For PLGA-NPs alone sample, (c) Size Distribution for PLGA-NPs – BSA sample, (d) Zeta potential ForPLGA-NPs – BSA sample, (e) Size Distribution for PcDNA3.1-VEGF-PLGA-NPs (Polyplex), (f) Zeta potential for PcDNA3.1-VEGF-PLGA-NPs complex.

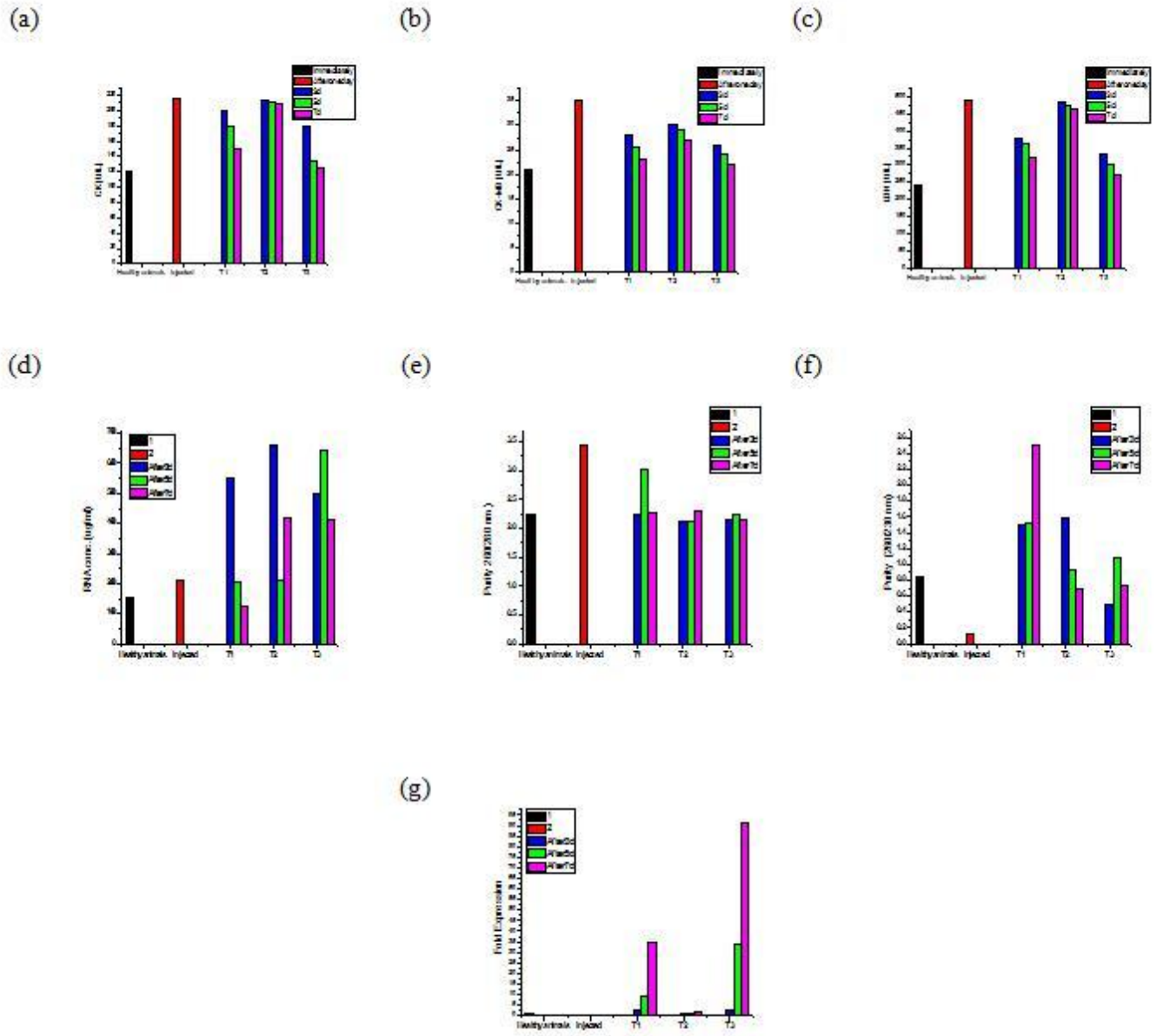

Figure 4: Graphical representation of, (a) CK (U/L) levels, (b) CK-MB (U/L) levels, (c) LDH (U/L) levels, and Comparison between studied groups regarding, (d) RNA concentration (ug/ml) (e) RNA Purity 260/280 nm, (f) RNA Purity 260/230 nm, (g) fold expression.

4.3. Histopathological finding of heart tissue:

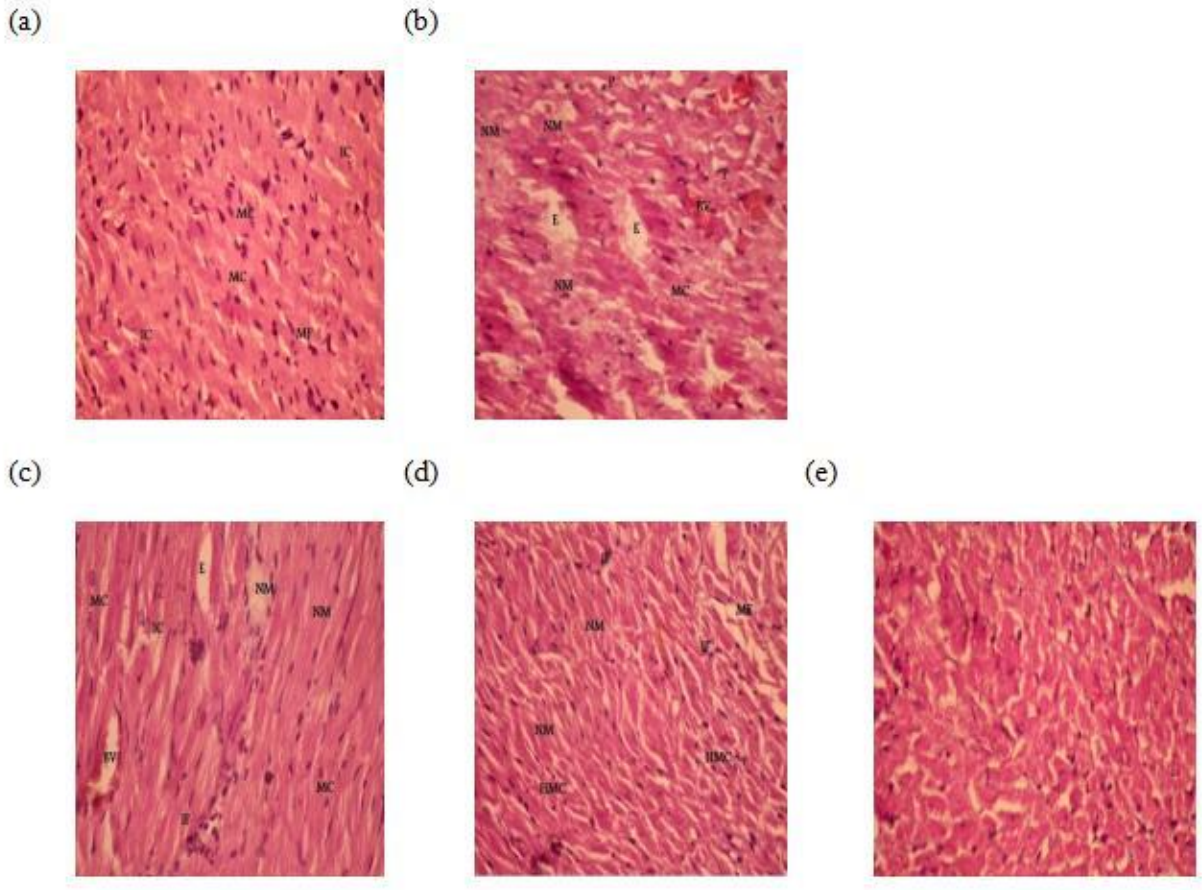

Figure 5: (a) Structure of myocytes of normal rat heart, (b) High power Paraffin section photomicrograph of myocardial infarcted rat group heart, (c) High power for T1 rat group heart showing some healthy myocytes, (d) High power for T2 rat group heart showing increased necrotic myocytes, (e) High power for T3 rat group heart showing higher healthy myocytes.

5. Discussion

Acute myocardial infarction takes place upon occlusion of one of the coronary Vessels, due to atherosclerotic plaque, resulting in an ischaemic region of myocardium.[31]

Nanotechnology is the understanding and control of matter at dimensions between 1 and 100 nm, which helps in prevention and treatment of various diseases.[32]

Gene therapy is a useful technique using genes that prevent or recover diseases, this allow treating of disorders by inserting a gene in cell of patients instead of drugs. This takes place by replacement of mutant gene causing disease with healthy genes.[33]

Gene transfering of angiogenic growth factors includes vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF), resulting in angiogenesis enhancement and functional recovering of ischemic tissues.[34]

Non-viral approaches began with delivering naked plasmid DNA, they are easier in manufacturing, purifying, chemically modifying, and can be scaled-up more than viral vectors.[35]

Cationic substances are polymers and lipids can be used as systems for non-viral gene transfer. Condensation of plasmid DNA carrying negative charge with positively charged cationic polymers or lipids can reduce plasmids size which enter cytoplasm and bind to cell membrane carrying negative charge and endocytosis occurs.[36]

Human VEGF family consists of 5 related glycoproteins VEGFA, VEGFB, VEGFC, VEGFD, and PIGF (Placental Growth Factor) which are

interact with family of 3 receptors: VEGFR1, VEGFR2 and VEGFR3.[37]

PLGA is biodegradable, biocompatible and FDA approved for biomedical applications, so PLGA is used in drug delivery systems.[38]

Nanoparticle formulated using PLGA polymers are a new delivery system for genes due to their sustained release characteristics, and they are able for protecting DNA from endolysosomal degradation.[39]

PLGA polymer is biocompatible, biodegradable. Polymeric nanoparticles are used as pharmaceutical dosage form of proteins and peptides. Many methods are applied in preparing polymeric nanoparticles, as emulsification-evaporation method.[40]

Biodegradable PLGA show properties for biotechnology through their biocompatibility and their authorization by the Food and Drug Administration (FDA) for drug delivery.[41]

Encapsulating peptide or protein by PLGA-NPs takes place by water-oil-water (w/o/w) emulsion technique.[42]

6. Conclusions

Non-viral gene delivery systems using plasmid DNA vectors are showing great promise in treatment of many diseases including Myocardial infarction.

Development of targeted PLGA nanoparticles carrier to a plasmid carrying genes such as VEGF has great benefit in gene expression in cardiac myocytes.

Gene targeting using biocompatible PLGA-NPs results in patient treatment of various diseases as myocardial infarction.

The gene transfer complex (Polyplex) "PcDNA3.1-VEGF-PLGA-NPs" in non-viral gene delivery showed highest expression level in mammalian myocytes, so it is mostly effective therapeutic material to obtain recovered myocardium and improving cardiac function.

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Conflict of Interest

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