Novel Delivery Systems of Therapeutics

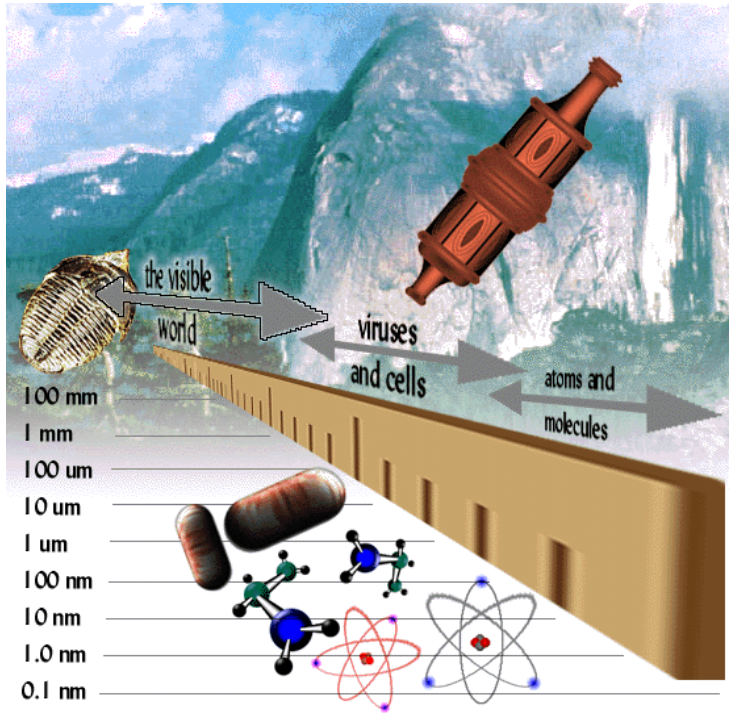
“Cytokines, mRNAs, Small Molecules”

Yasin Oduk, Ph.D., CEO & Founder NanomediGene LLC

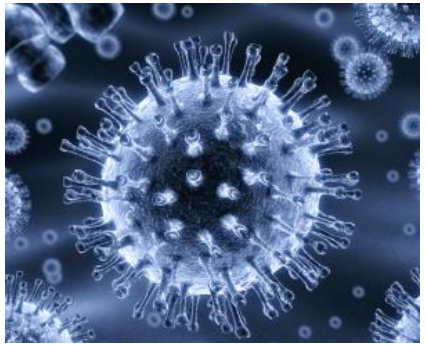
Bio2Device Talk

01/12/2021

OUTLINE



- Drug Delivery Systems
- Nanoparticles for drug delivery



- Targeted Drug Delivery
- Challenges with insoluble small molecules

- VEGF Nanoparticles Repair Heart after Myocardial Infarction
- MicroRNA Nanoparticles
- Hydrogels

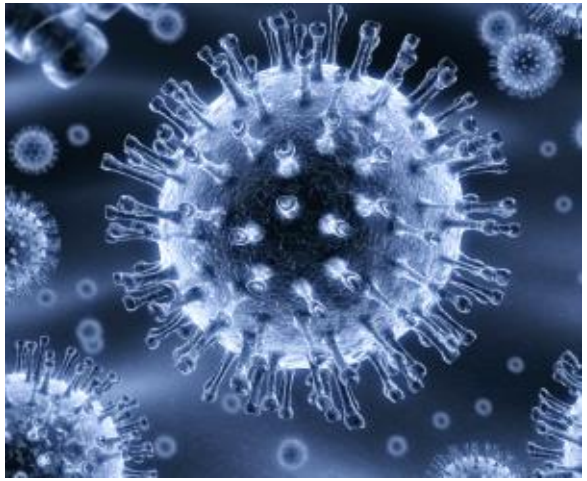
Drug Delivery Systems

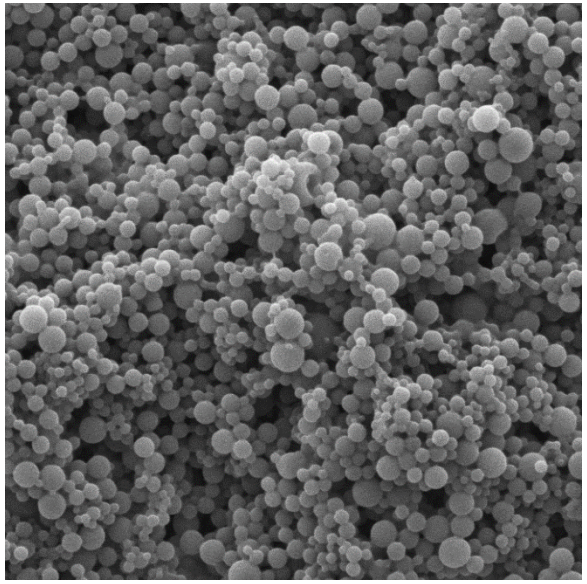
- Microparticles
- Nanoparticles
- Hydrogels
- Biofilms
- Protein Conjugation
    - Controlled Release
    - Layer by Layer release
    - Targeted delivery
    - Local Delivery

Nanoparticles for Drug Delivery

Nanoparticles can be easily tailored and synthesized to suit for drug delivery.

- Size
- Charge
  - Negatively charged particles are cleared faster than positively charged particles.
  - Surface charge effects how nanoparticles interact with cells, whose membranes are usually negatively charged.
- Biodegradability
- Controlled Release





http://nanogloss.com/category/nanoparticles/#axzz4YiAwFsRj

Pfizer & Moderna Covid 19 vaccine

- mRNAs packed into lipid nanoparticles
- Protect fragile mRNA molecules
- Stealth
- polyethylene glycol (PEG)

Sizes of Some Molecules

Substance	Approximate molecular mass (g/mol)[1]	Effective molecular radius (nm)[1]	conc. in ultrafiltrate / conc. in blood plasma[1]
sodium	23	0.1	1.0
potassium	39	0.14	1.0
chloride	35.5	0.18	1.0
water	18	0.15	1.0
urea	60	0.16	1.0
glucose	180	0.33	1.0
sucrose	342	0.44	1.0
polyethylene glycol	1,000	0.70	1.0
inulin	5,200	1.48	0.98
lysozyme	14,600	1.90	0.8
myoglobin	16,900	1.88	0.75
lactoglobulin	36,000	2.16	0.4
egg albumin	43,500	2.80	0.22
Bence Jones protein	44,000	2.77	1.0
hemoglobin	68,000	3.25	0.03
serum albumin	69,000	3.55	<0.01

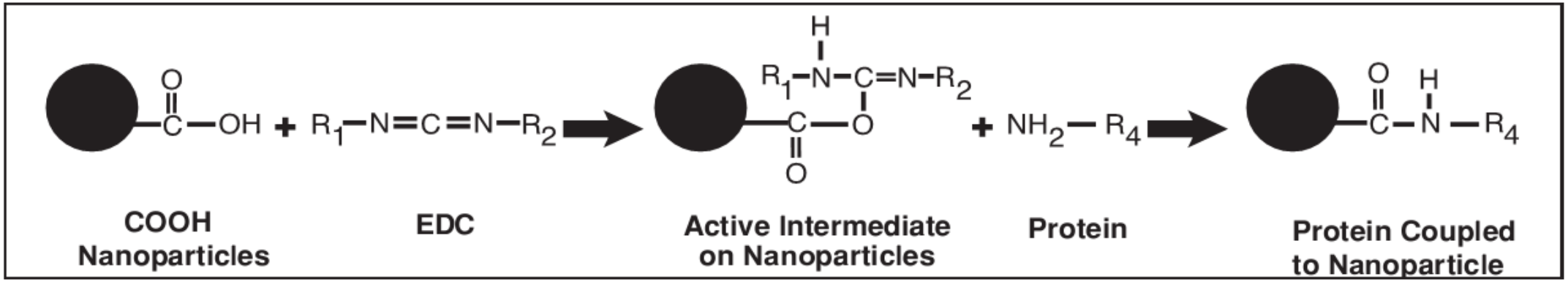
https://en.wikipedia.org/wiki/Table_of_permselectivity_for_different_substances

Targeted Delivery

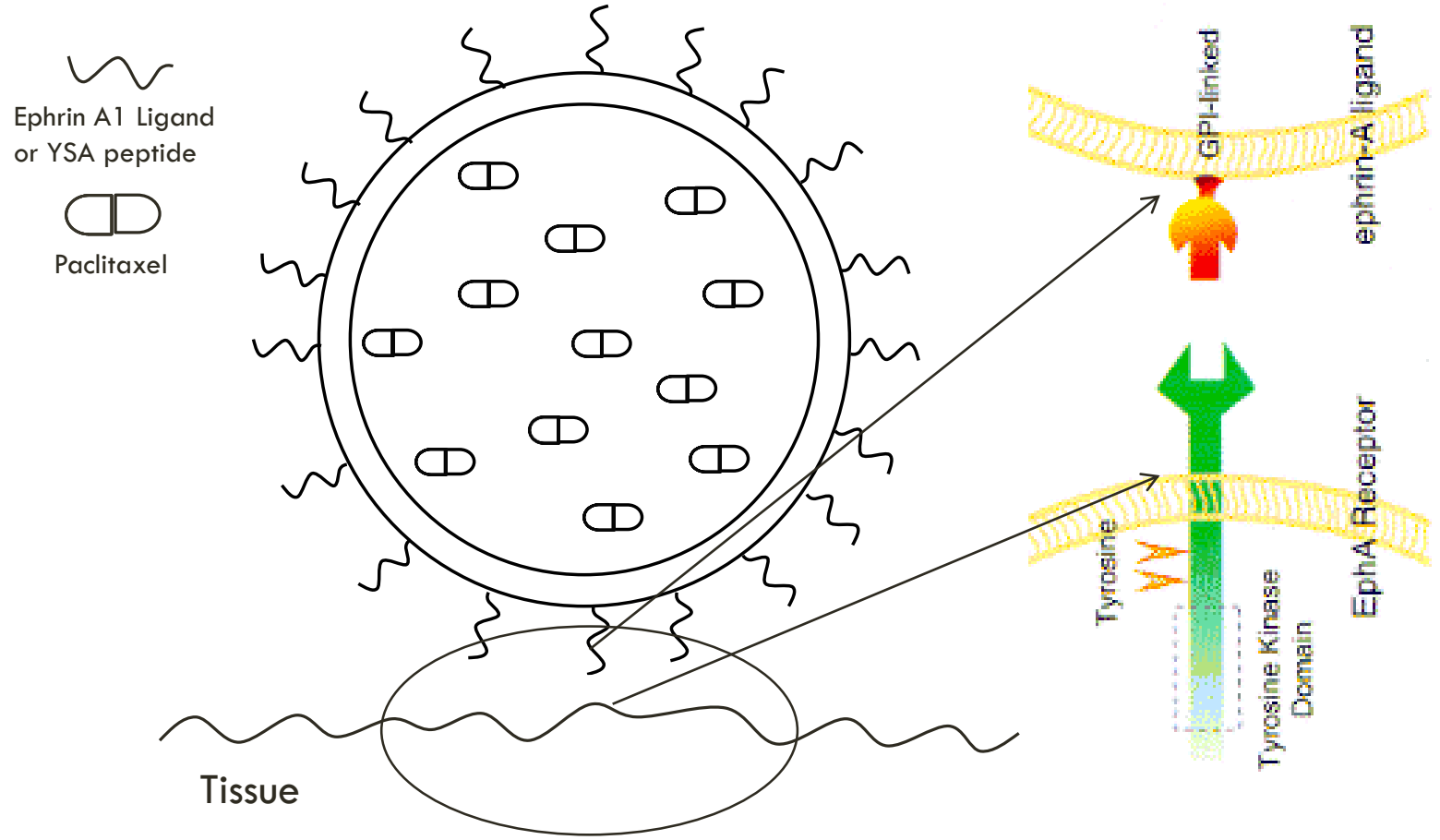
Targeting agents such as ligands, antibodies could be conjugated onto surface of PLGA nanoparticles

Carboxyl (COOH) groups on PLGA NP can be used for covalent coupling of proteins by

- Activating the carboxyl groups with a crosslinker (EDC)
- The EDC reacts with the carboxyl group
- Reacts with primary amines on the protein

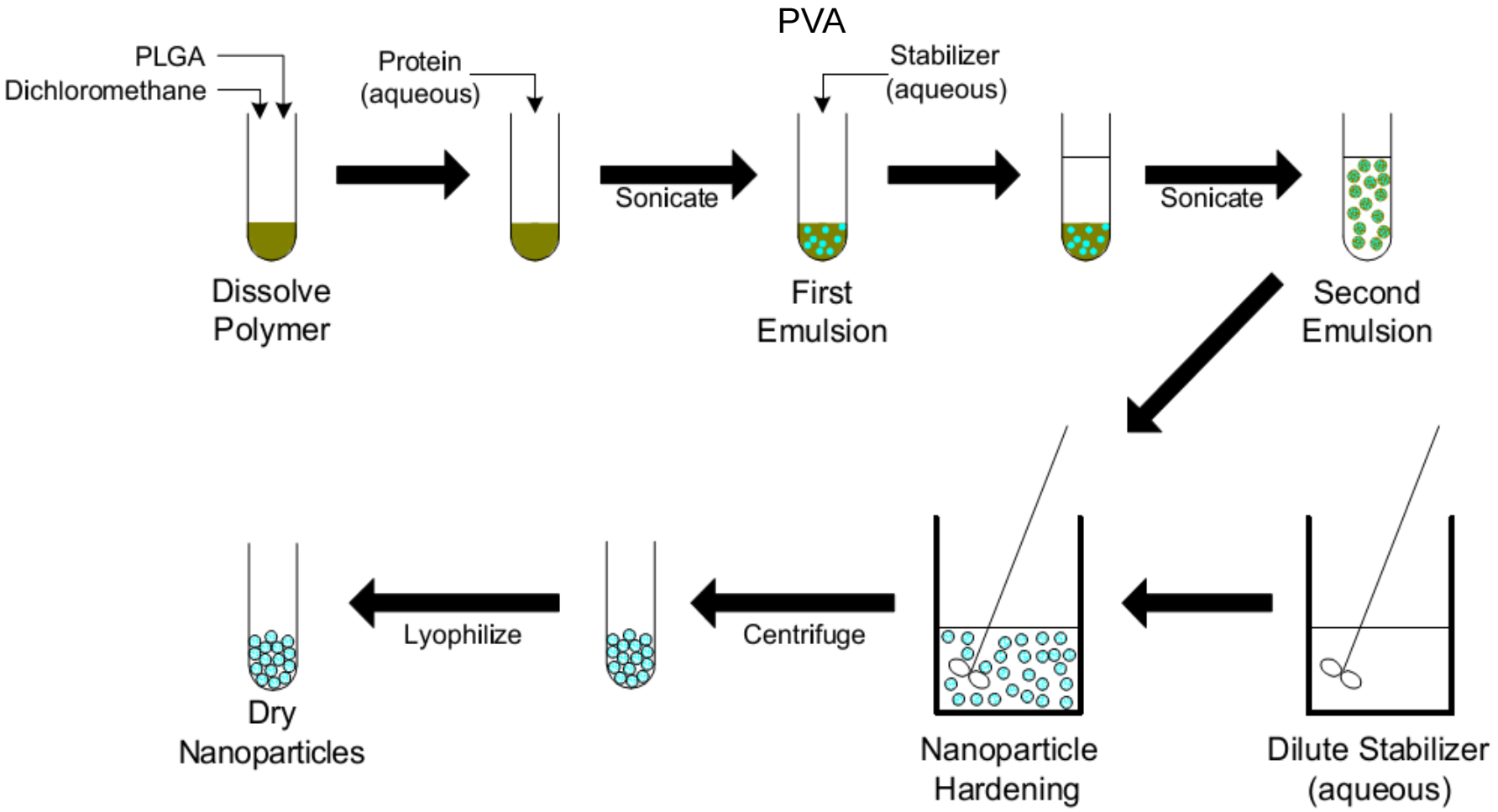


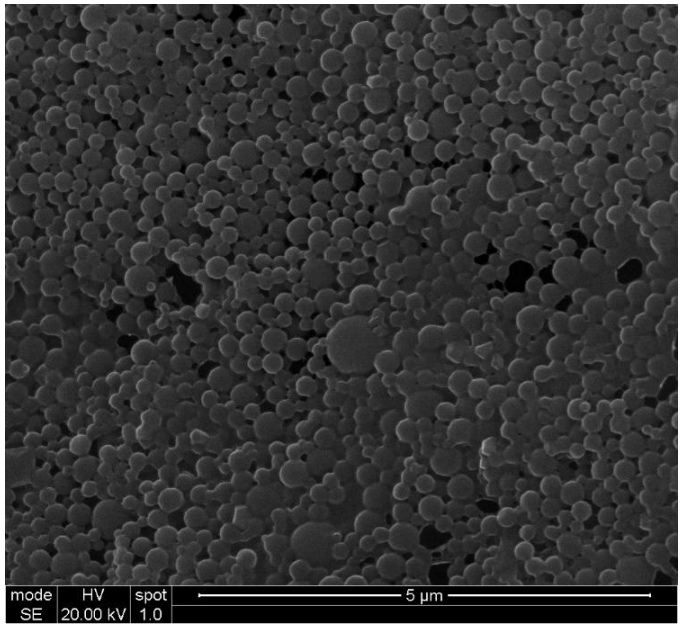
Mechanism of Targeting Nanoparticles



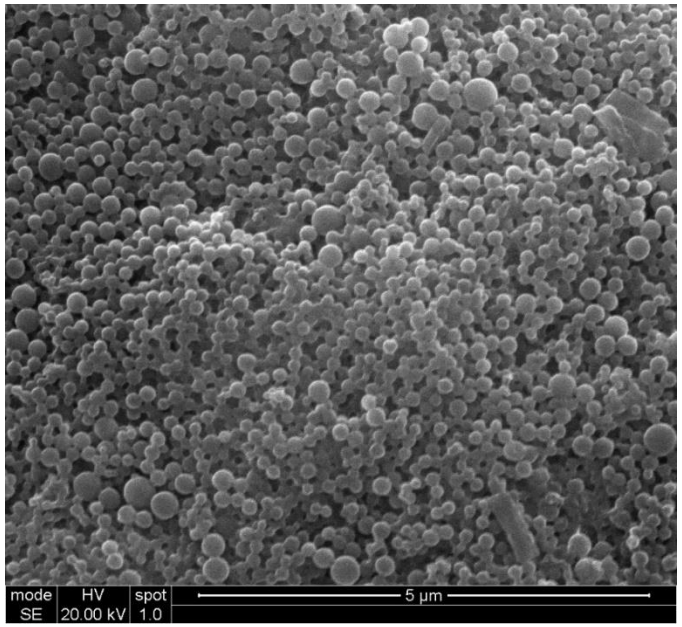
Preparation of PLGA Nanoparticles

Double Emulsion: [Water-in-oil-in-water (W/O/W)]

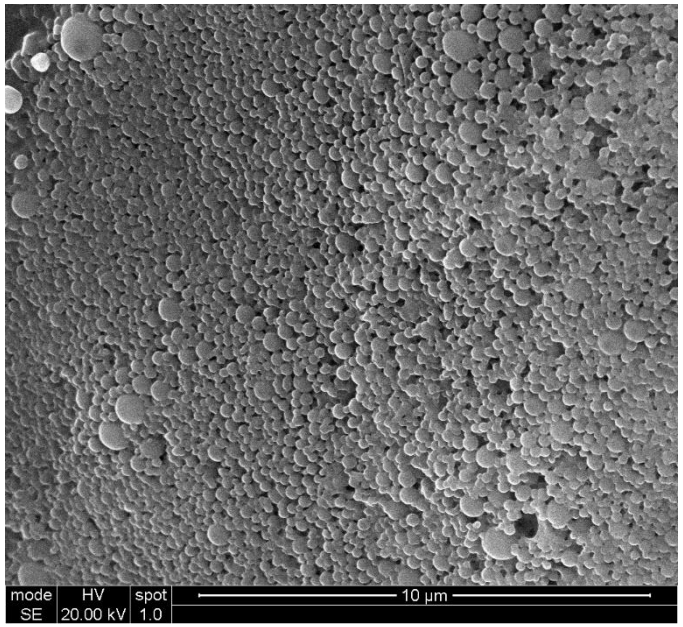




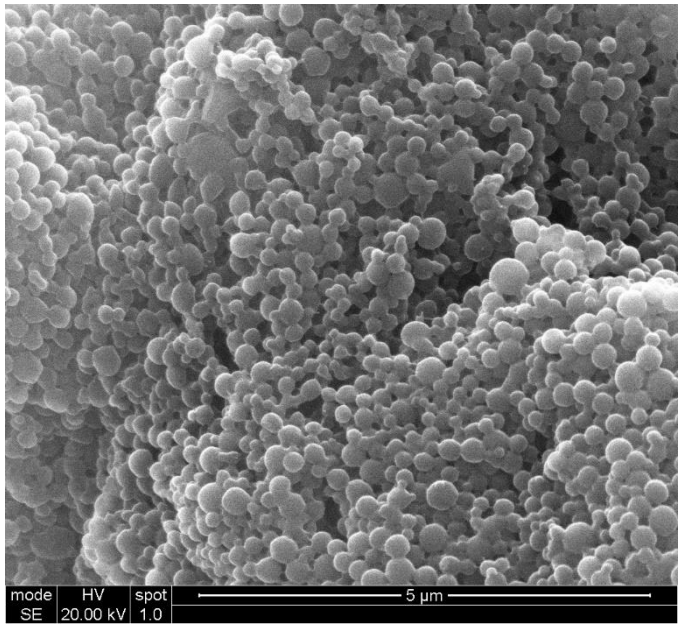
E-2 2 20 kx se_052 Size 192 nm



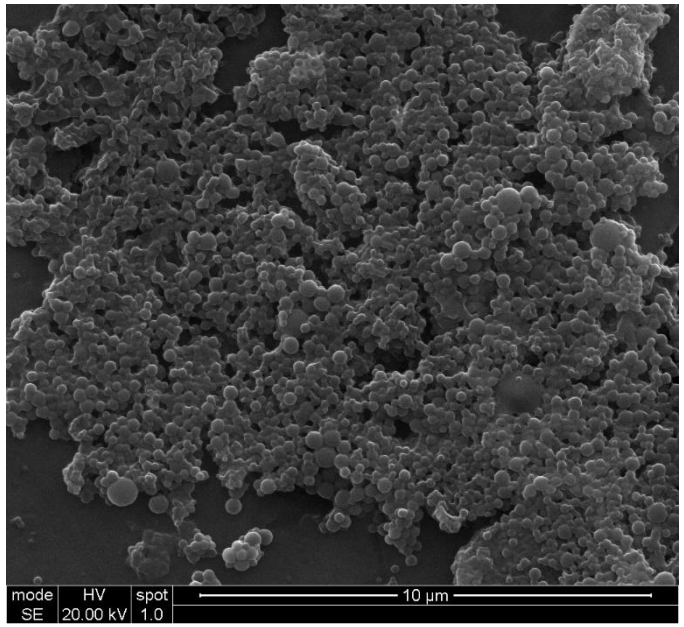
E-3 3 20kx se_053 Size=147 nm



C-3 5 10kx se_023 Size=215 nm

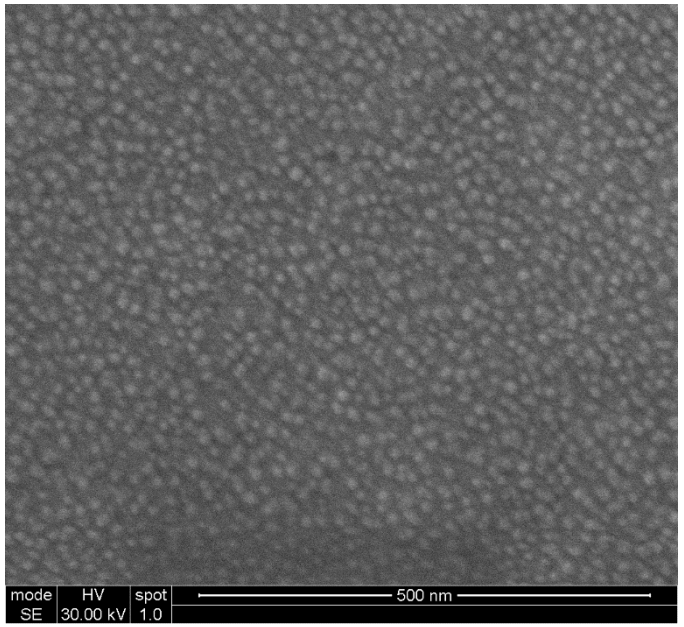


C-4 6 20kx se_035 Size=215 nm



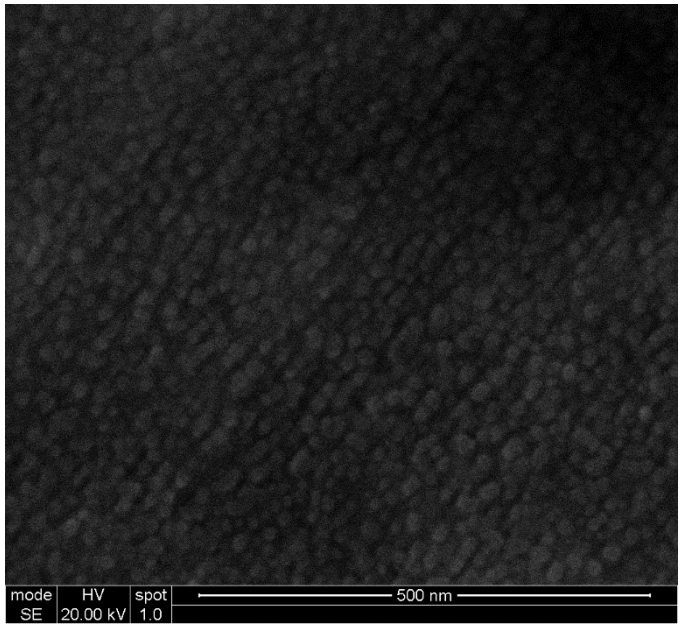
C-5 7 10kx se_40 Size=251 nm

Ultra Small Particles



E2 NPs (E-1) 1-3

Nanoparticles <10 nm could enter the nucleus,
whereas larger ones found only in the cytoplasm.

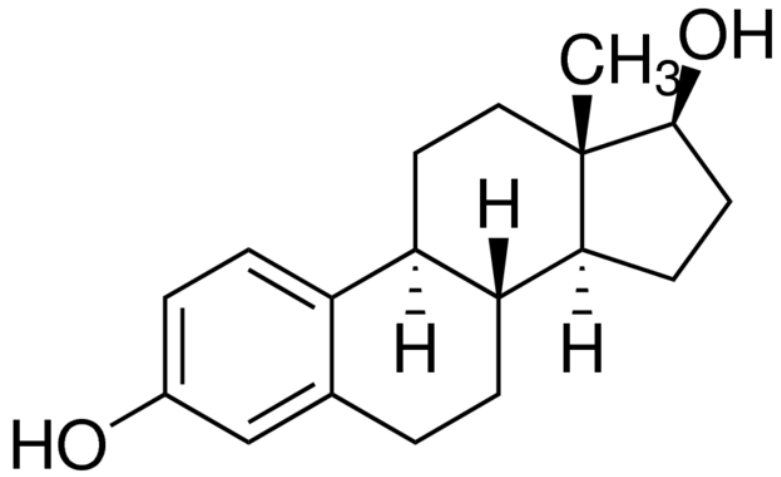


E2 NPs (E-2) 2-11

Huo, 2014, Ultrasmall Gold Nanoparticles as Carriers for Nucleus-Based Gene Therapy Due to Size-Dependent Nuclear Entry, ACS Nano

E2 (β-Estradiol)

- **Molecular Weight: 272.38**
- **Formula: $C_{18}H_{24}O_2$**
- **Dissolves in absolute Ethanol**



E2 Release in Literature

Investigating Sustained-release Nanoparticles for Pulmonary Drug Delivery, Liu, Yao-Tsapis, Nicolas-Edwards, David A / Journal of Controlled Release, 2003

The estradiol concentration appeared to remain constant throughout the sampling period at a level that suggests that all of the estradiol was released immediately upon incubation. It may be that the estradiol was localized along the outside of the PLGA-estradiol

First human experience with the 17-beta-estradiol-eluting stent: The estrogen and stents to eliminate restenosis (EASTER) trial

JACC Vol. 43, No. 6, 2004
March 17, 2004:1118–21

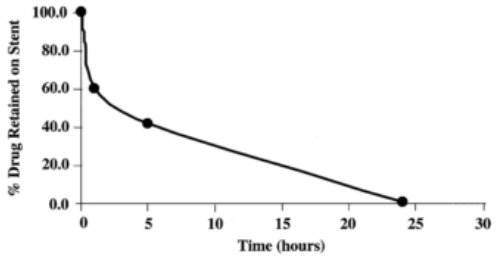


Figure 3. Graph showing the phosphorylcholine polymer local delivery pharmacokinetics of 17-beta-estradiol. Total drug delivery is completed within the first 24 h.

Preparation and characterization of estradiol-loaded PLGA nanoparticles using homogenization-solvent diffusion method, Esmaeili, F, Daru, Journal of Faculty of Pharmacy, 2008

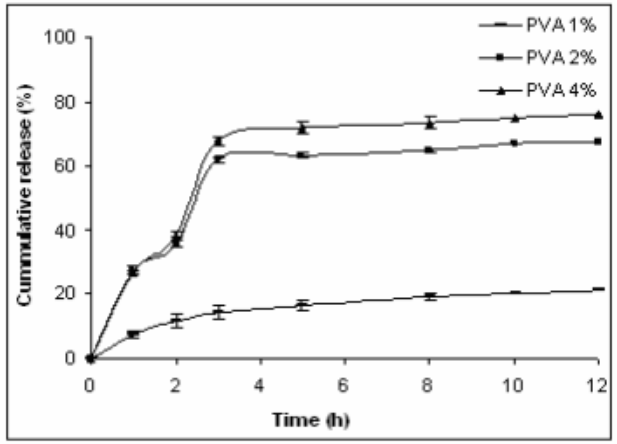


Figure 3. Effect of PVA concentration on the in vitro estradiol release from PLGA nanoparticles (n=3), error bars show the average amount of drug released ± SE

Estradiol loaded PLGA nanoparticles for oral administration: Effect of polymer molecular weight and copolymer composition on release behavior in vitro and in vivo, Mittal, 2007, Journal of Controlled Release

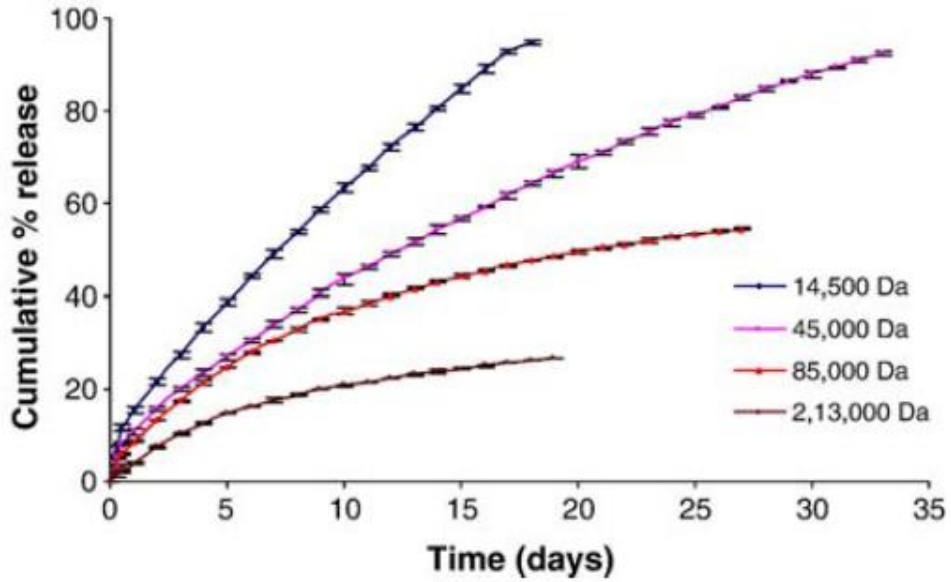
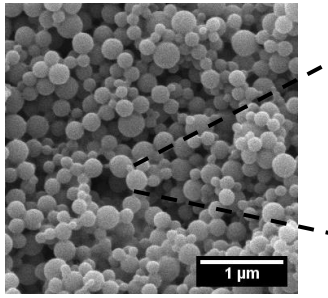
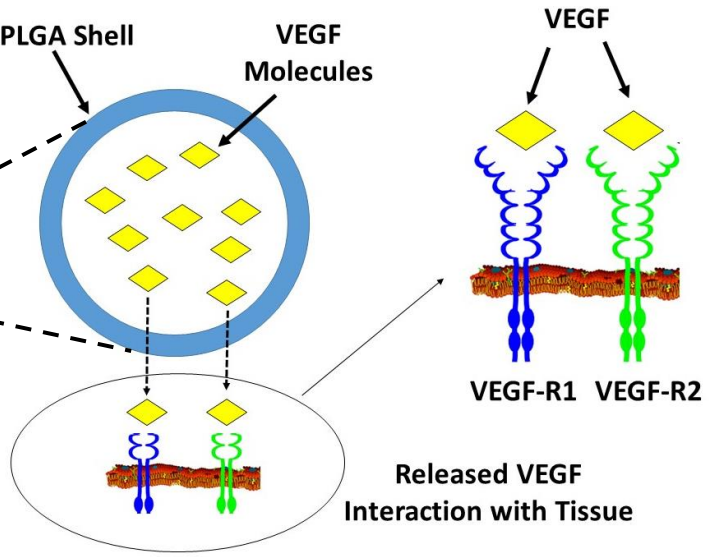


Fig. 1. *In vitro* release profiles of estradiol loaded PLGA (50:50) nanoparticles of different molecular weights with DMAB as stabilizer in pH 7.4 phosphate buffer. Data points shown are mean ± standard deviation ($n = 3$).

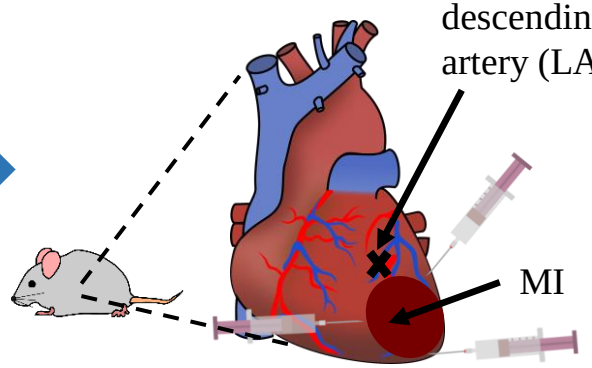
VEGF Nanoparticles Repair Heart after Myocardial Infarction

Sustained release of VEGF from PLGA nanoparticles improves LV function and vascular density and reduces infarct size in mouse heart post MI.

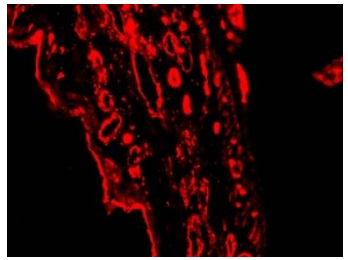




MI was induced and mice were injected with 3 doses VEGF nanoparticles:

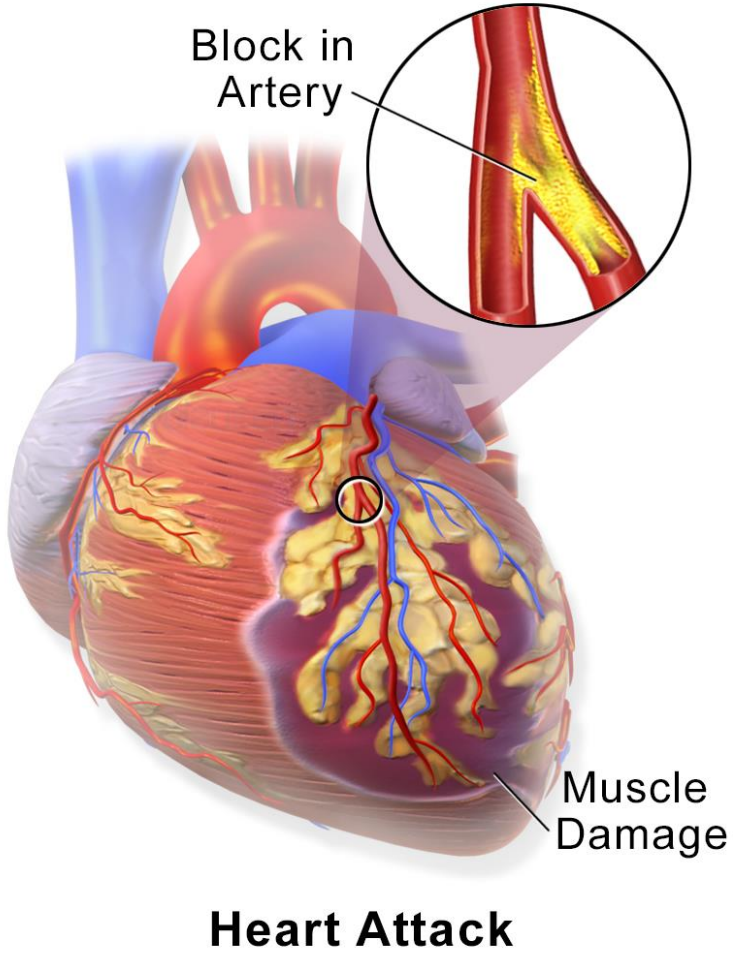


Improved;
- Cardiac functions
- Vascular density



Reduced;
- HW/BW
- Infarct size

Myocardial Infarction: MI



https://upload.wikimedia.org/wikipedia/commons/f/fb/Blausen_0463_HeartAttack.png

VEGF

- **VEGF:** Vascular endothelial growth factor
  - ➢ Promotes cardiac vascularization

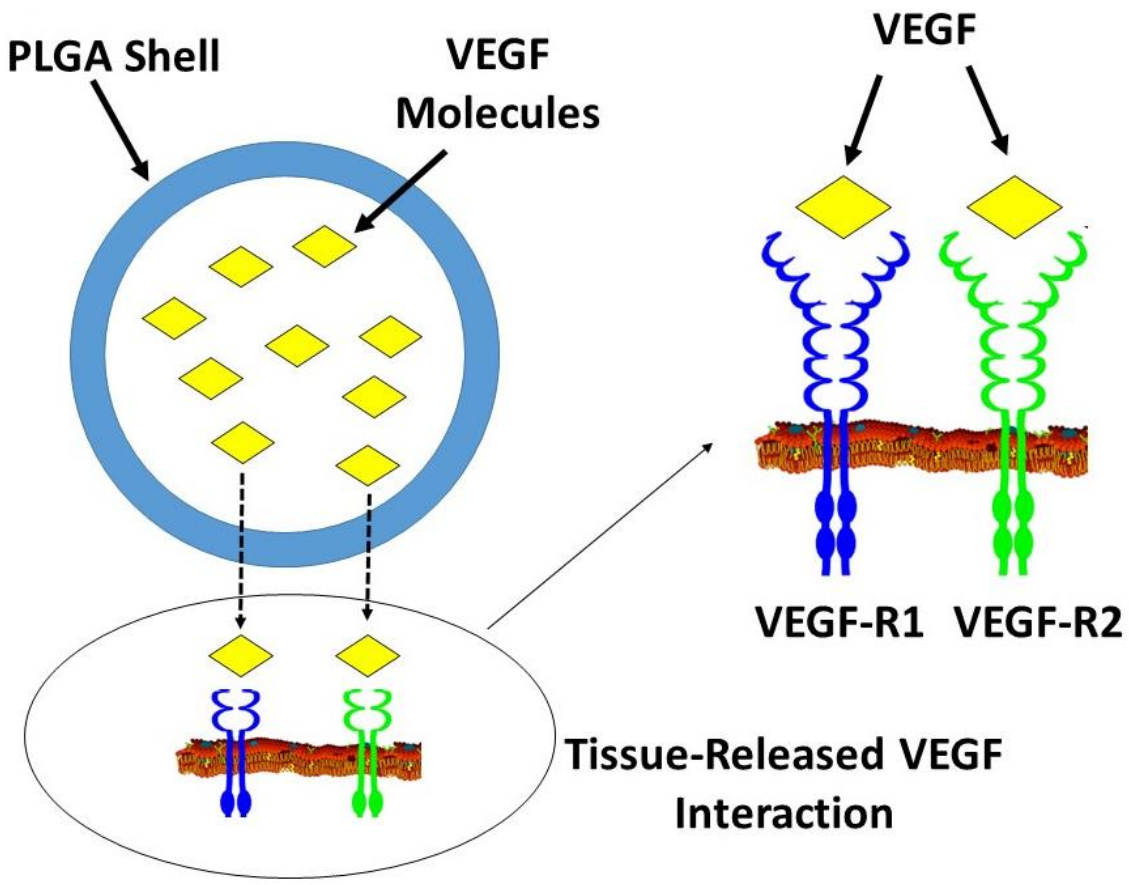
- **MI and Vascular Growth:** Treatment of myocardial infarction is crucially dependent on
  - ➢ **Vascular growth**

Problem:

- Human clinical trials have been disappointing due to
- Short in vivo half-life of VEGF (~30 min)
  - Requires excessive injection and
  - Extremely high doses.
- Undesired vascularization in non-target sites
- Denaturation of proteins

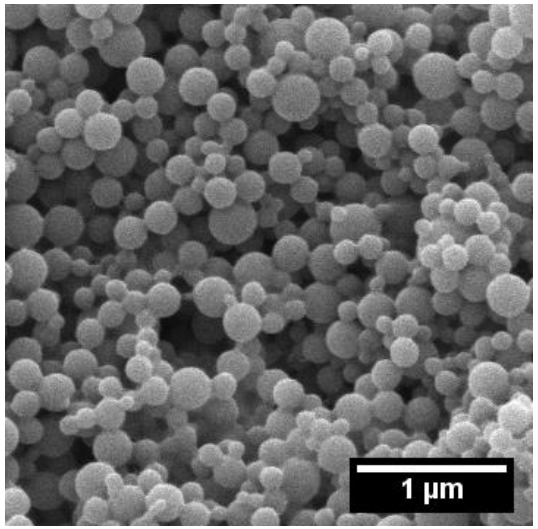
Hypothesis

- Protect VEGF from in vivo environment
  - Encapsulating VEGF in PLGA nanoparticles will preserve the functional properties and improve the half-life of VEGF.
- Prolonged Release
  - Sustained release of VEGF from the nanoparticle would maintain required VEGF plasma levels avoiding excessive administrations and thus eliminating VEGF side effects.
- VEGF-containing nanoparticles could improve
  - Cardiac function,
  - Remodeling, and
  - Angiogenesis in mice after MI.

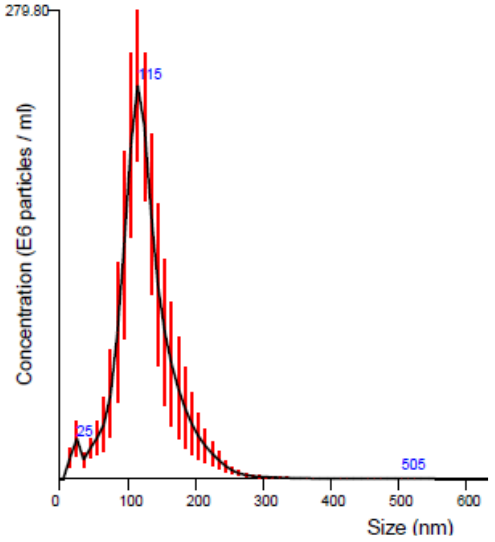


Characterization of VEGF Nanoparticles

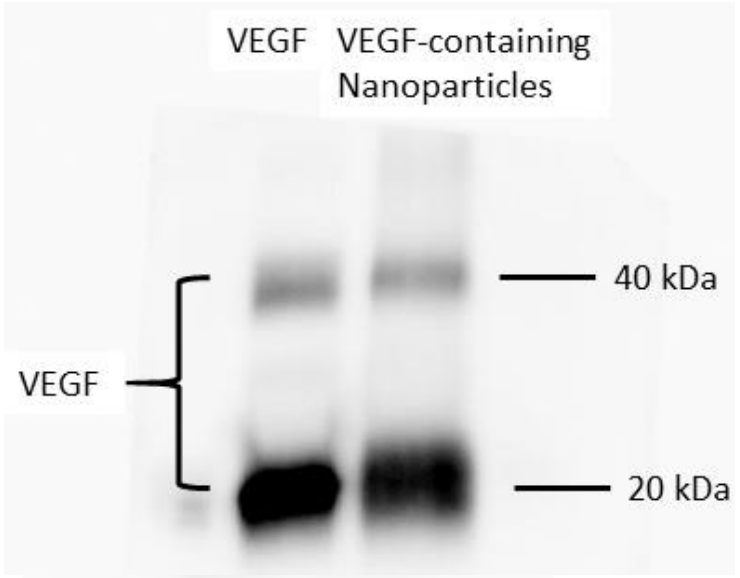
A.



B.



C.

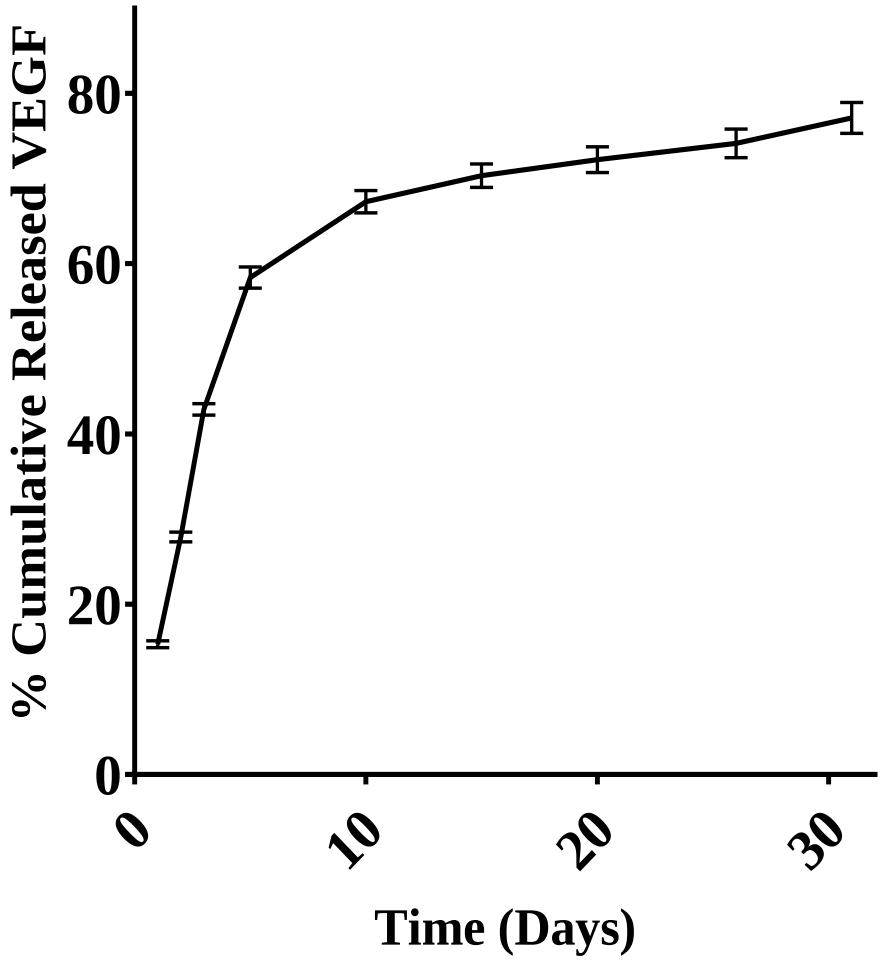


D.

Nanoparticle Contents	Size (nm)	Encapsulation Efficiency (%)	VEGF Concentration (ng/mg)	Surface Charge (mV)
Empty	115.6±1.3	NA	NA	–56.2±8.5
VEGF	113.1±5.2	53.5±1.7	107.1±3.3	–55.4±8.2

Human VEGF 165 Recombinant Protein
Mw: 19.2 kDa

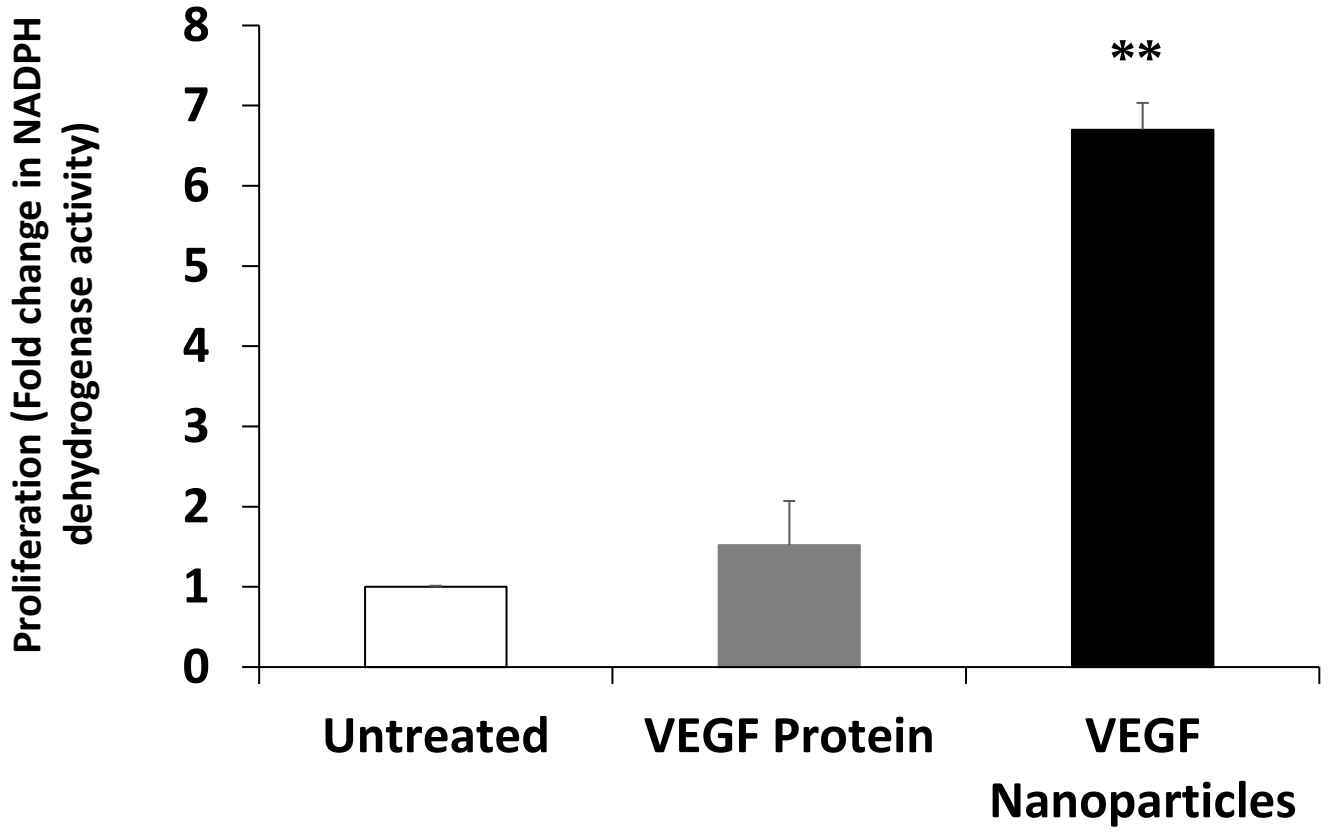
Release Profile of VEGF Nanoparticles



PLGA nanoparticles release measurable amounts of VEGF for up to 31 days *in vitro*.

Proliferation (MTS Assay)

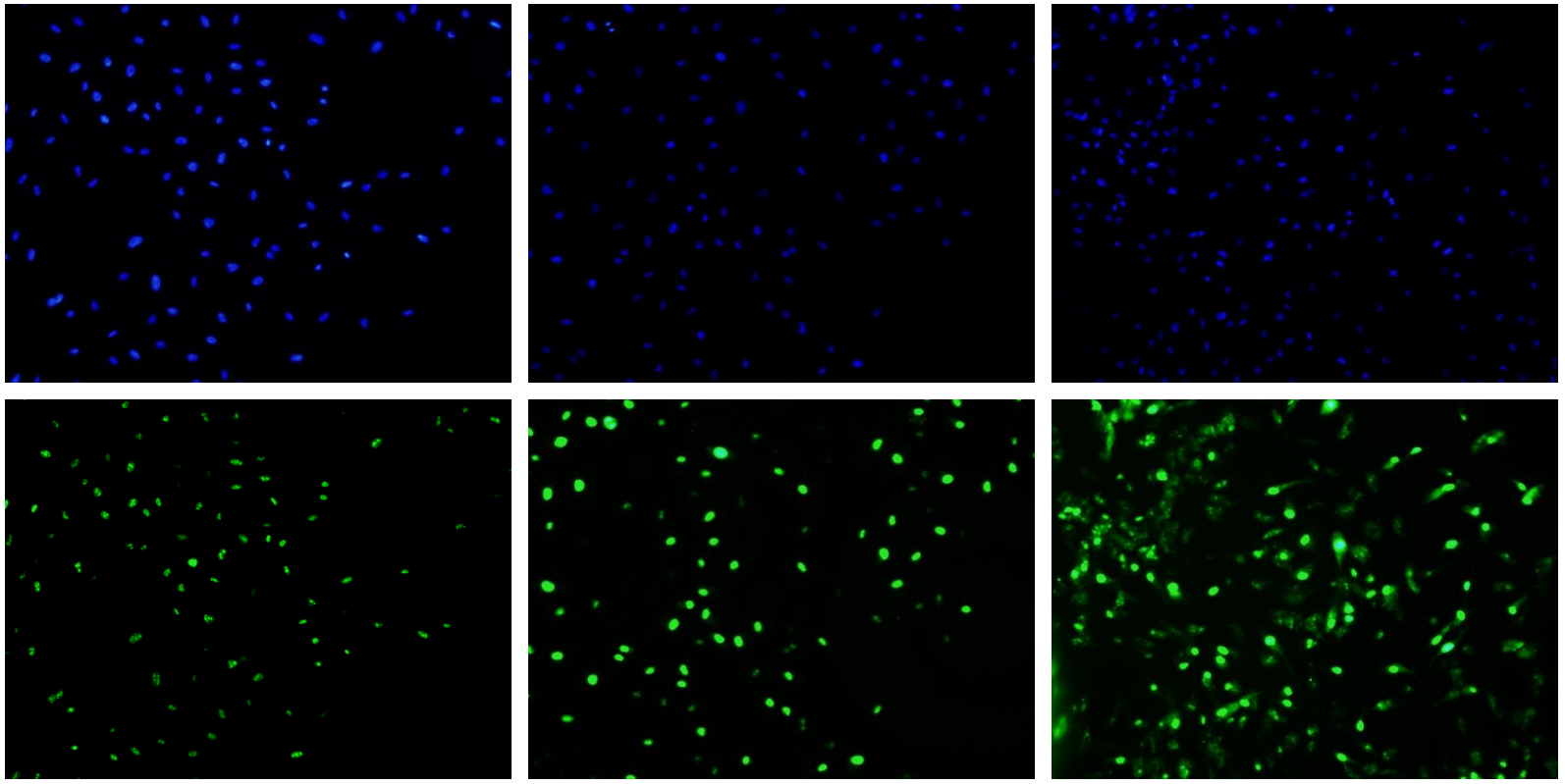
VEGF nanoparticles show greater potency in stimulating the pro-angiogenic activity of cultured HUVECs than free VEGF protein.

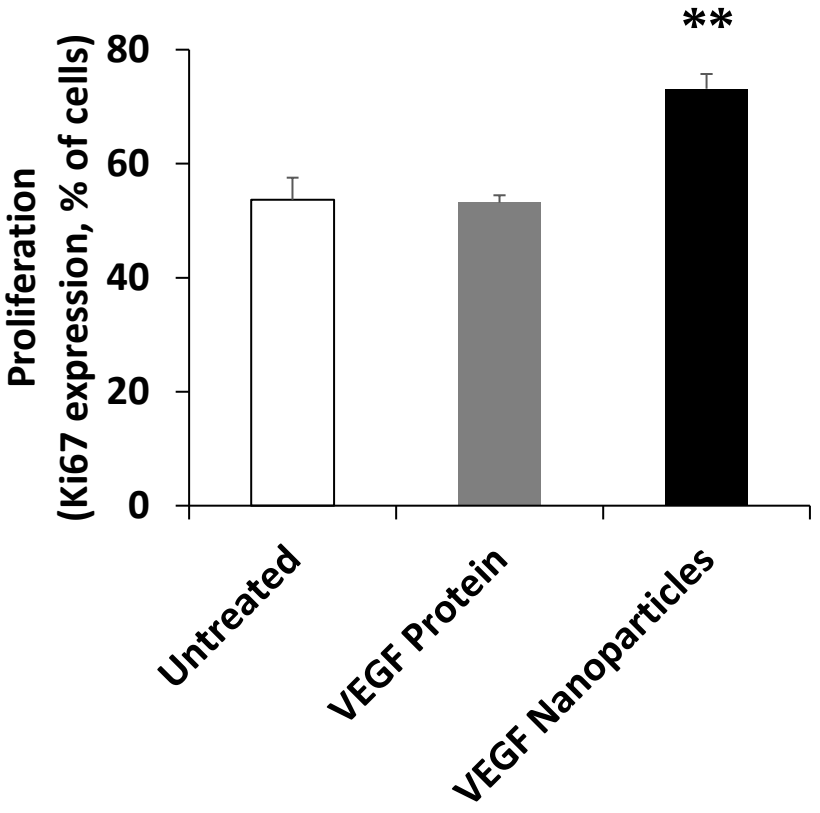


**P<0.05 versus Untreated and free VEGF protein

Proliferation (Ki-67 Staining)

(A) Untreated cells **(B)** VEGF Protein **(C)** VEGF Nanoparticles **(D)**



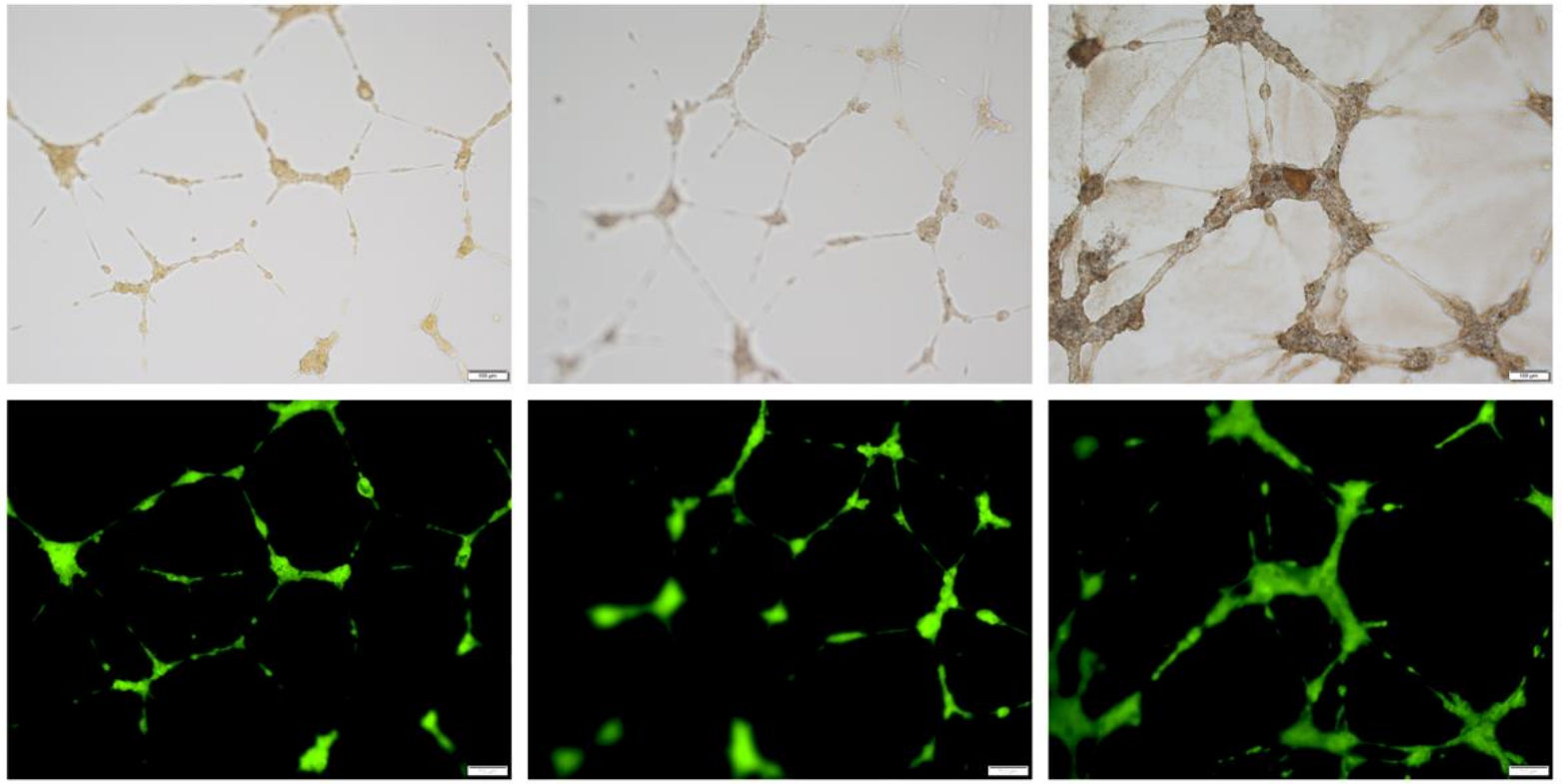


**P<0.05 versus Untreated and free VEGF protein

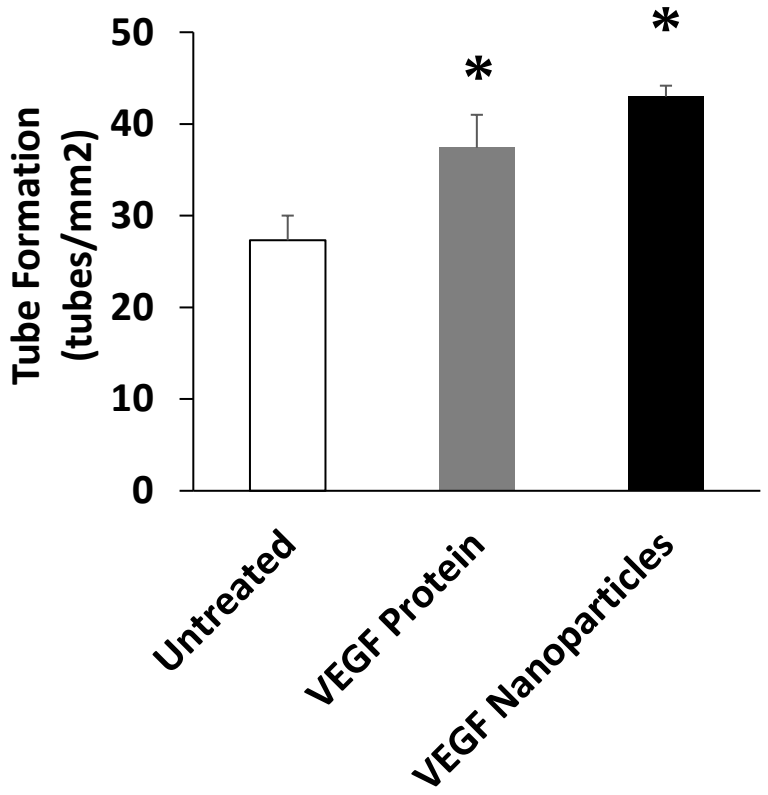
VEGF nanoparticles show greater potency in stimulating the pro-angiogenic activity of cultured HUVECs than free VEGF protein.

Tube Formation

(A) Untreated cells **(B)** VEGF Protein **(C)** VEGF Nanoparticles



(D)



*P<0.05 versus Untreated

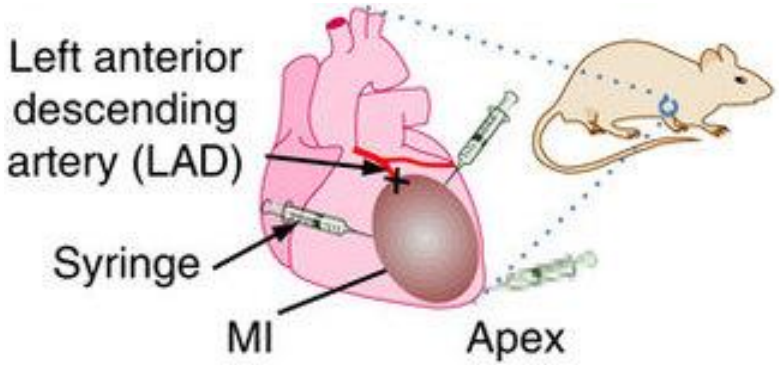
VEGF nanoparticles show greater potency in stimulating the pro-angiogenic activity of cultured HUVECs than free VEGF protein.

Animal Experimental Plan

- Echocardiography Scan

0 Week

- MI induced and mice were injected with VEGF nanoparticles:



1. **MI+NPLD**: 6 ng n=7
2. **MI+NPMD**: 2.4 ng n=9
3. **MI+NPHD**: 0.6 ng n=5

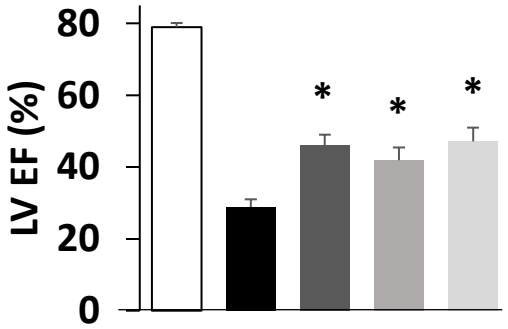
4. **MI:** Untreated Control n=8
5. **Sham,** n=5

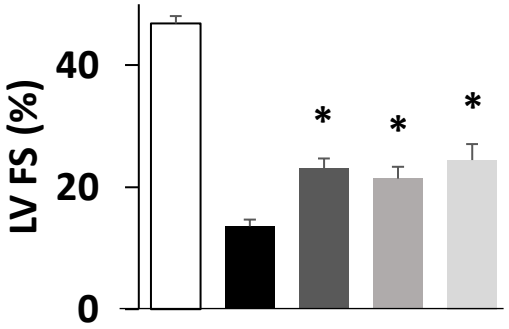
- Echocardiography Scan

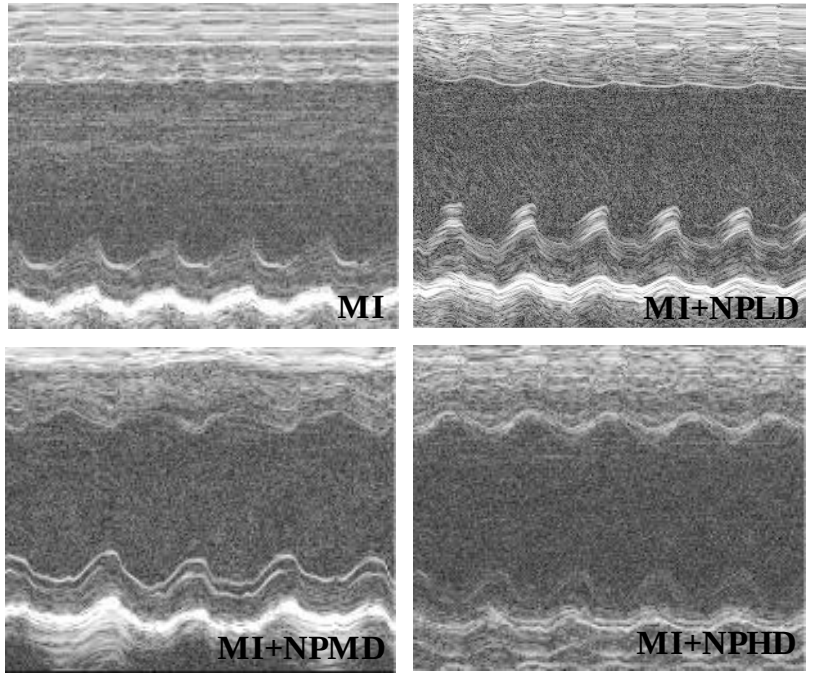
4 Week

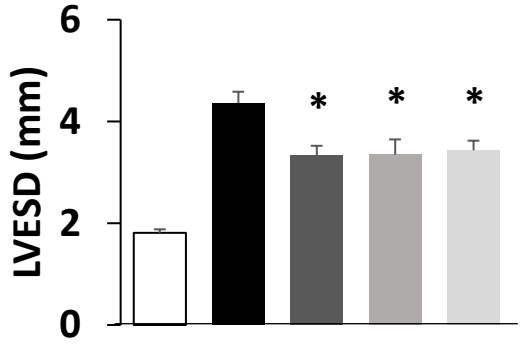
- Sacrifice and Tissue Analyses
  - Sirius-red– and fast-green–staining
  - CD31 Staining

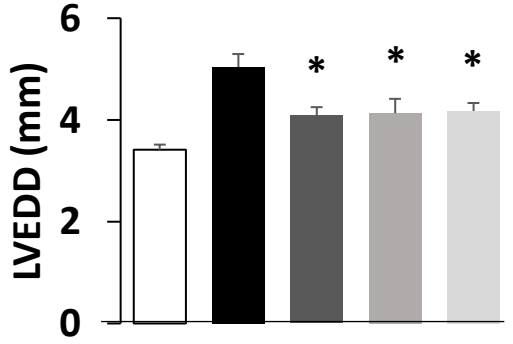
Cardiac Function by Echocardiography







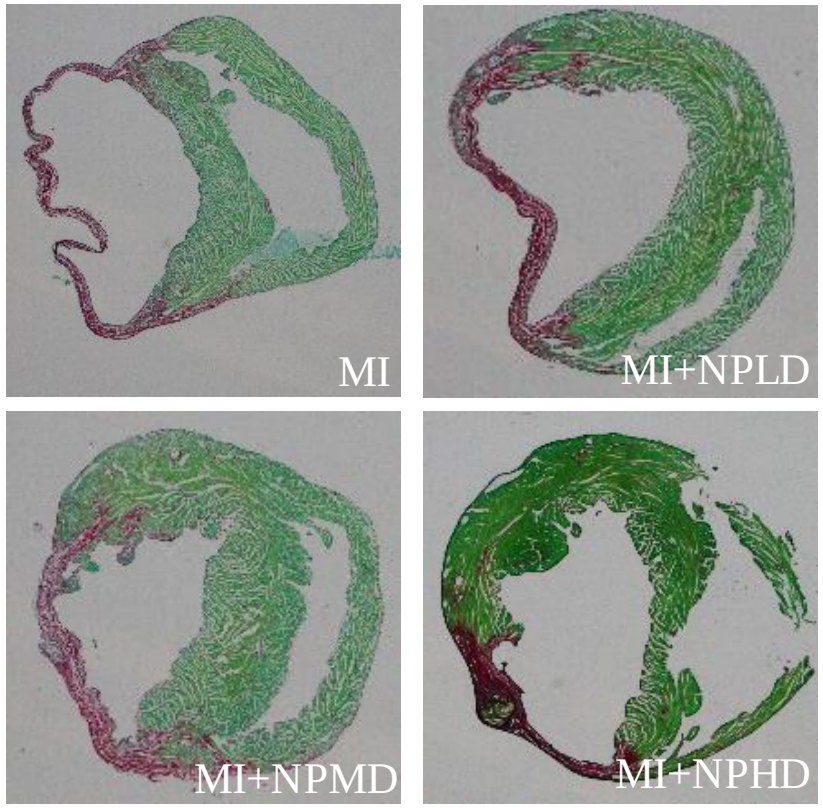


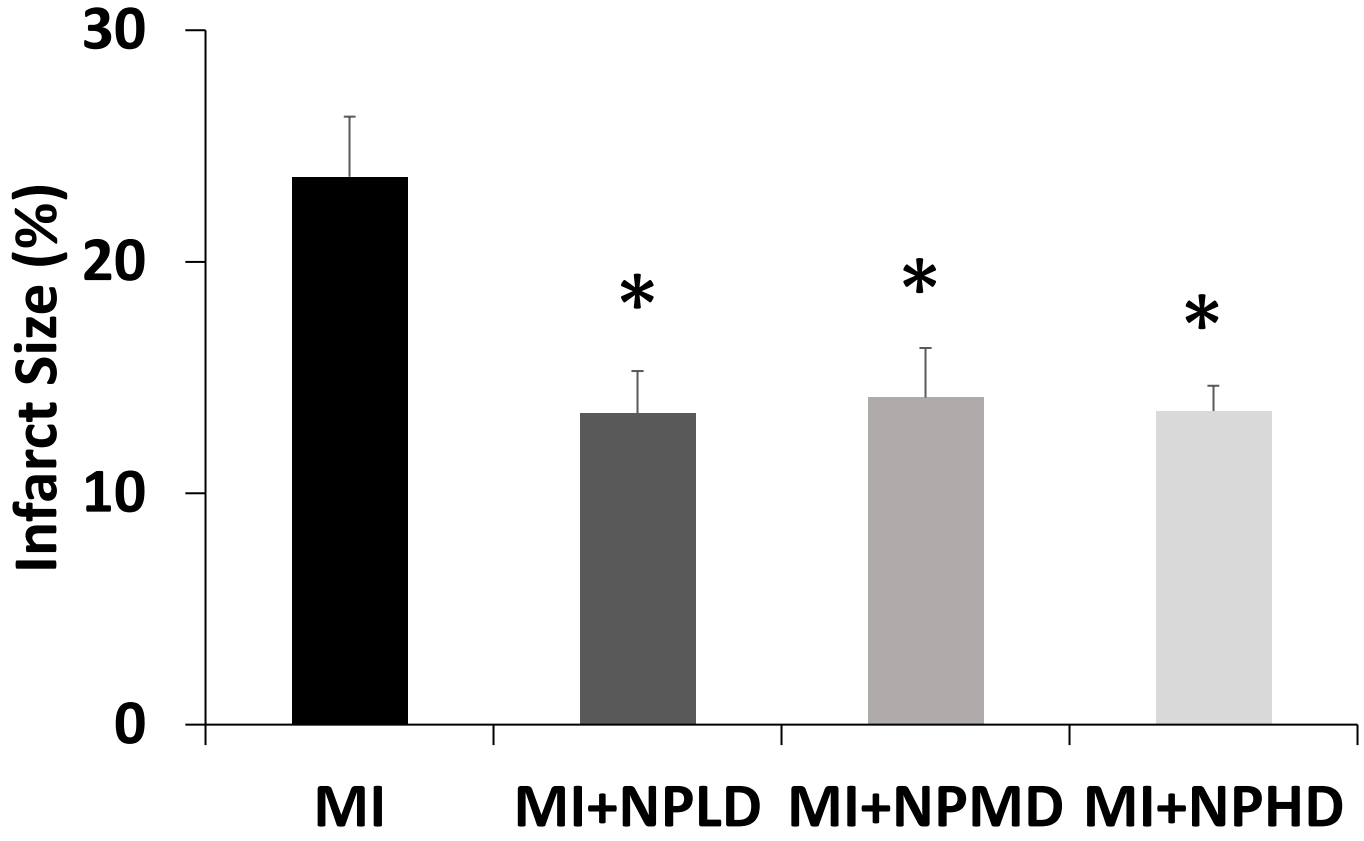


*P<0.05 versus MI and SHAM

□ SHAM ■ MI ■ MI+NPLD ■ MI+NPMD ■ MI+NPHD

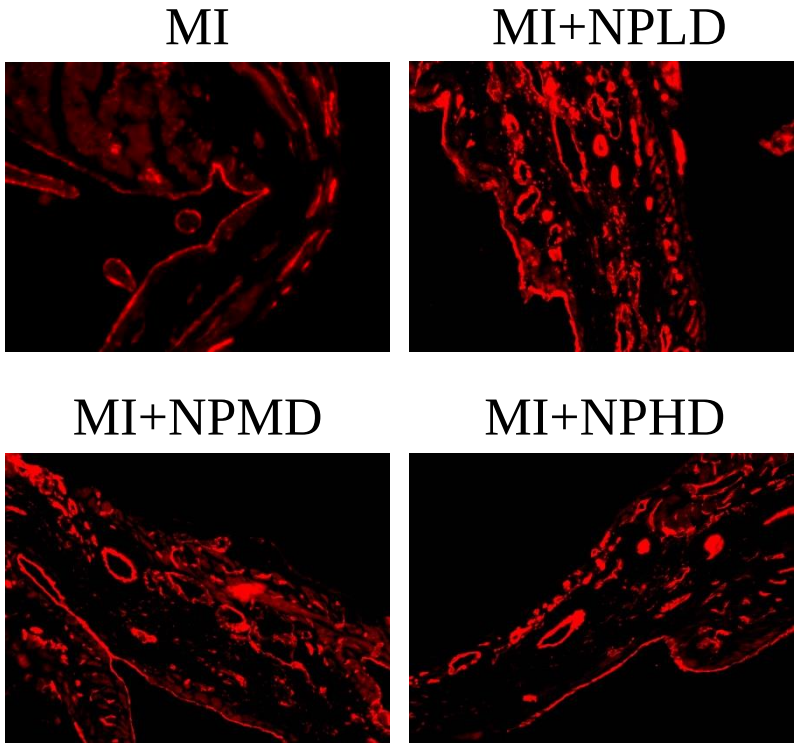
Infract Size

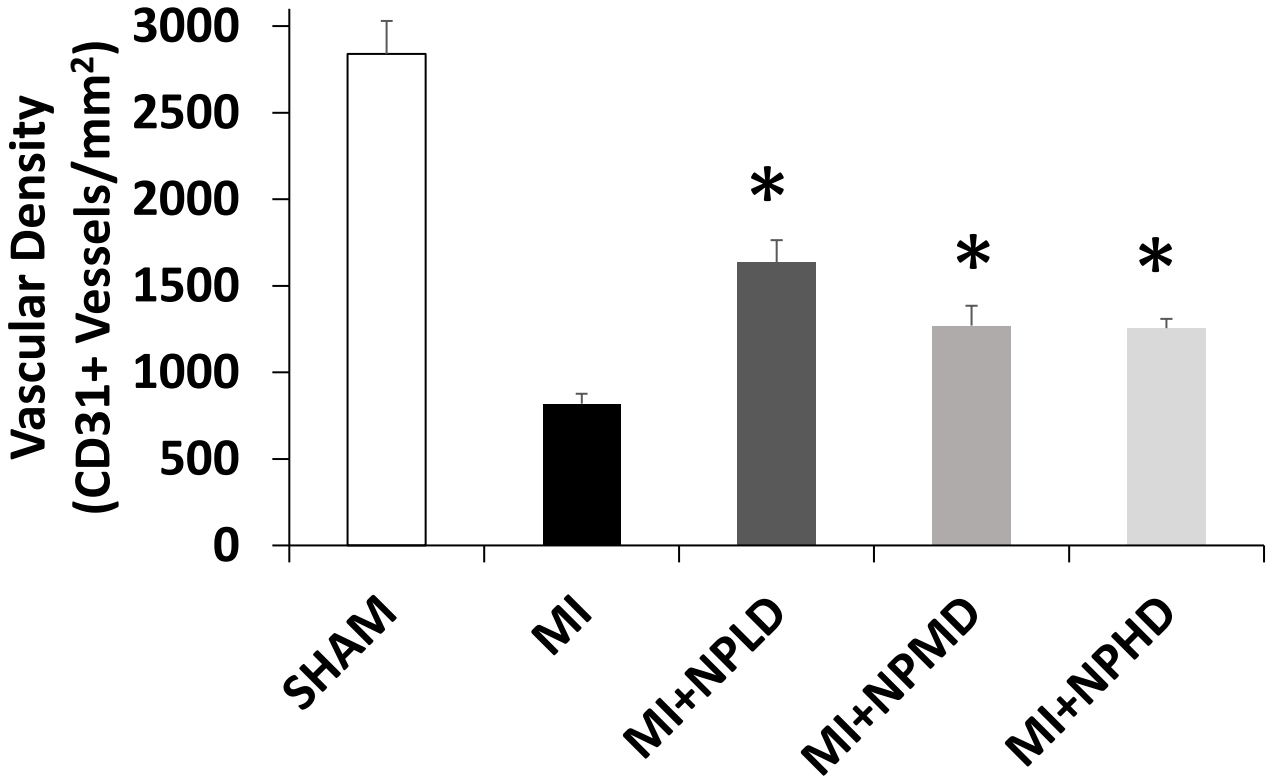




*P<0.05 versus MI

Vascular Density





*P<0.05 versus MI

Conclusion

VEGF Nanoparticles can protect VEGF inside polymer shell thus

➢ Improve half life of VEGF

VEGF Nanoparticles can provide sustained release thus

➢ Improve effectiveness of VEGF by;

- 7 fold better proliferation
- Improved Cardiac Functions
- Increased Vascularization in infarcted heart

Thus VEGF-PLGA nanoparticles can be considered as a promising drug delivery system to promote revascularization in the damaged myocardium of cardiac patients.

Thank You!

Questions?