Design of a targeted nanomedicine to diminish oxidative stress in atherosclerosis

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Statement of Purpose: Atherosclerosis is a very serious vascular diseases leading to thickening and hardening of vessel walls as well as loss of flexibility so that vascular occlusion happens. Vein constriction due to accumulation of lipid molecules on the main arteries results in myocardial infarction or ischmeia. Actually lipid layers on arteries (LDL-Low Density Lipoprotein) originate from the oxidation of lipid molecules and then it leads to inflammation at the site. Particularly drug therapies that are not targeted to damaged site lead to toxicity and side effects for healthy tissues and organs. So it becomes obvious to have a need for targeted therapies that deliver drug molecules only to site where disease conditions are seen. So in this research project, a targeted drug delivery system for the treatment of atherosclerosis was prepared by conjugating drug molecules (lipoic acid) to nanoparticles with a slow and sustained release profile in the form of nanogels which is made out of biocompatible and biodegradable (Poly(ethylene glycol) (PEG)) chains. Based on the findings about VCAM-1 adhesion molecule on damaged arteries are over-expressed compared to healthy ones, anti-VCAM1 antibody was attached to periphery of nanogels to direct drug molecules to only damaged part of arteries.

Methods: Nanoparticles were prepared by free-radical based gellation procedure by using dimethacrylate-functionalized PEG polymers under UV light (365 nm). 3 different molecular weight of PEG chains (2, 6 and 10K) were evaluated under same gellation conditions. Dilution factor, time and temperature for nanogel formation procedure were optimized as well as drug content being as 5, 10 and 20% by weight. A novel monomer with three drug molecules attached via a hydrolysable linker and one methacrylate group was successfully synthesized. Ultra-filtration by centrifugation method was used to remove any impurities and excess drug molecules. Cy5 dye was conjugated to surface of amine functionalized nanogels for cellular internalization studies. On the other hand, excess amine moieties were linked to anti-VCAM-1 antibody for targeting purpose via HATU/DIPA coupling. Cytotoxicity of drug-loaded nanogels were investigated not only against L929 mouse fibroblasts but also HUVEC cells at both resting state and activated by LPS, an endotoxin to create an inflammation environment.

Results: LA3MA monomer was successfully synthesized as three lipoic acid drug molecules linked by ester bonds and a reactive methacrylate group in its structure and obtained in high purity (99.58%) with 76% yield (Figure 1a). This drug-containing multivalent monomer was characterized for its chemical structure and functional groups by ^{1}H NMR and FT-IR spectroskopies. LC-MS and LC-MSMS analysis were used for determining its purity and molecular weight. On the other side, varying molecular weight of PEGDiMA polymers (Figure 1b) were utilized to obtain PEG-based nanogels under UV light (365 nm) in the presence of a photoinitiator, (I2959).

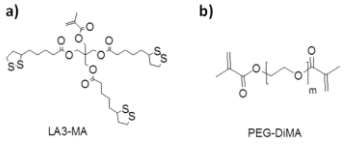
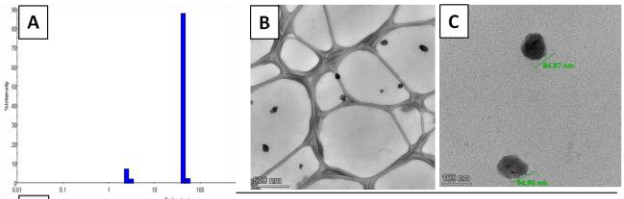


Figure 1. Chemical structures of LA3MA (a) and PEGDiMA ((b).

Nanogel formation procedure was optimized for its polymer:initiator ratio, time, temperature, solvent type and dilution factor. It was found out that the nanogels with a size ~100 nm, required for nanoparticles to penetrate into injured tissue better, was obtained with PEGDiMA polymer with 6 kDa, at room temperature and in 10mM pH7.4 PBS solution. Polymers used at a concentration of 1mg/300µL solvent with 1:50 polymer:photoinitiator ratio by weight resulted in nanogels as a monomodal shape and with low PDI value. Later on, LA3MA monomer was included into the gellation with 5, 10 and 20% feed ratio by weight and drug-loaded nanogels were obtained to bear drug encapsulation efficiency as 76.21, 85.53 and 87.94, respectively. Drug loading capacity values for nanogels as the corresponding feed ratios were calculated as 6.57, 15.58 and 24.19, respectively.



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LA3-MA content (%)	Size (nm)	PDI (%)	Intensity (%)	Mean zeta potential (mV)
0	42.8	4.4	90.5	-8.1
5	43.2	18.1	85.4	-0.2
10	37.0	11.8	98.2	1.5
20	56.7	19.5	92.6	8.8

Figure2. (A) Size distribution peak for PEG-based nanogel, (B and C) its TEM images, (D) Size and zeta potential details of prepared nanogels.

Moreover, PEG-based nanogels were synthesized as 2% amine containing versions, which were used to conjugate Cy5 as imaging agent (400 µg/mL) and anti-VCAM-1 antibody as 1-2 in average MABs per nanoparticle.

References:

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