Using Curcumin Nano-Lipid Particles in a Therapeutic Approach

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Abstract

Curcumin (*Curcuma longa)* is a plant-based polyphenol known to have several medicinal properties. Although several promising effects of using curcumin in clinical trials have been observed, its overall medicinal qualities are still limited due to low bioavailability. In order to increase the bioavailability, we are embedding curcumin within Nano-Lipid Particles (both curcumin telodendrimer discs and curcumin tNLPs).

Telodendrimer nanolipoprotein particles (tNLPs) are discoidal self-assemblies containing lipids and apolipoproteins which can be used as a vehicle to carry proteins and other small molecules to the cell. Telodendrimer NLPs have been used to increase the bioavailability of drugs, and provide an ideal platform to increase curcumin bioavailability. The generation of tNLPs can be accomplished using several methods; such as cell-free assembly and *in-vitro* assembly.

Curcumin telodendrimer discs (curcumin telo-discs) are a nano-lipid mixture of lipids, curcumin, and telodendrimer that acts as the basis for the curcumin tNLP reaction. Using the curcumin telo-disc as the starting additive, we demonstrate that we can purify properly formed curcumin tNLPs via affinity columns and size-exclusion chromatography (SEC). Here, we show that with two separate methods: a cell-free expressed method and *in-vitro* assembly, we can demonstrate that curcumin can be successfully incorporated and purified within the tNLP particle in a single reaction tube.

Introduction

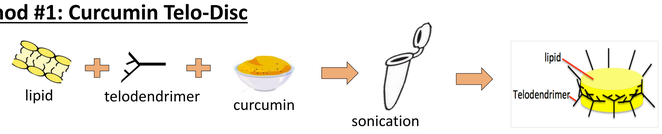
Nano-lipid particles act as a platform for delivering and increasing the bioavailability of drugs as well as other small molecules into the cell. Both telodendrimer discs (telo-discs) and telodendrimer nanolipoprotein particles (tNLPs) have the potential to increase cellular uptake of the "cargo" with which they hold. By loading the curcumin molecule onto telo-discs and tNLPs, we provide a novel platform to increase curcumin bioavailability. Upon curcumin loading, we named these nano-lipid particles "curcumin telo-discs" and "curcumin tNLPs", respectively.

We illustrate three main methods we developed to form curcumin nano-lipid particles. Method 1 outlines the curcumin telo-disc assembly. In brief, lipid, telodendrimer, and curcumin are mixed and sonicated in an Eppendorf tube. After sonication, curcumin telo-discs have been formed.

Method 2 illustrates the cell-free production of curcumin tNLPs. In this method, we combine curcumin, lipids, telodendrimer and ApoA1 plasmid to the reaction tube. Upon overnight incubation and shaking, curcumin tNLPs form spontaneously.

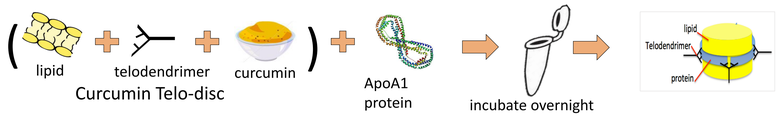
Method 3 demonstrates an *in vitro* production of curcumin tNLPs. Using the best-condition from the cell-free assembly, we begin this procedure with curcumin telo-disc formation. After curcumin telo-disc has been formed, we add in ApoA1 protein and incubate overnight to form spontaneous curcumin tNLPs.

Method #1: Curcumin Telo-Disc



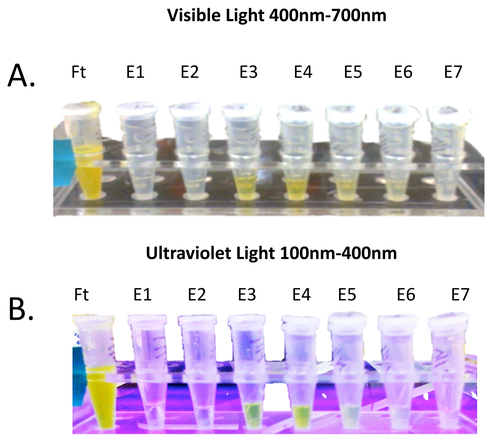
Method #2: Cell-Free Curcumin tNLPs

Method #3: *In Vitro* Curcumin tNLPs



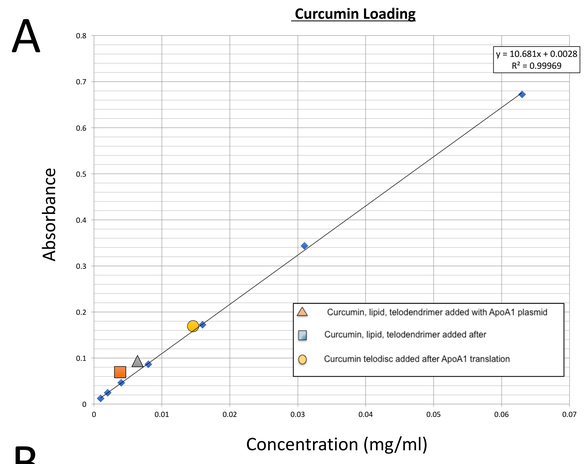
There are two variations to the procedure above:
1) Sonicate for 30 minutes before overnight incubation.
2) Sonicate for 30 minutes before overnight incubation, and add more curcumin the next morning.

Curcumin-tNLPs can be affinity purified after assembly



The pictures represent the elutions from affinity purification using column chromatography to purify the nanoparticles. A) Illustrates the color of the elutions containing curcumin in visible light, which show a yellow color indicating the presence of curcumin. B) Illustrates the color of the elutions containing curcumin in UV light, which indicates the solubility of the curcumin associated with the nanoparticle.
We pooled elutions that have intense yellow coloring, indicating increased curcumin concentrations, for further analysis.

Cell-free expression can be used to create curcumin loaded nanoparticles



B

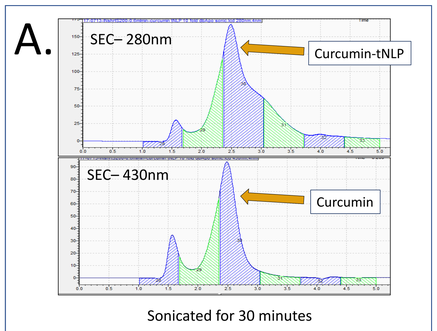
	Loading Capacity
Curcumin tNLP E2/ E3 7/7/16	1.75%
Curcumin tNLP E3/ E4 7/14/16	2.8%
Curcumin tNLP (curcumin telo disc added to cell free reaction) 7/18/16	7.3%

Loading Capacity Equation:

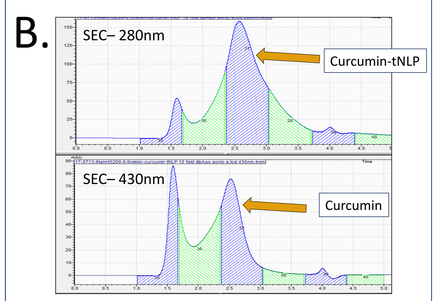
$$\frac{\text{mg Curcumin}}{\text{mg Apolipoprotein A1}}$$

We used cell-free approaches for making nanoparticles (**Methods 1 & 2**). The standard curve created uses the absorption of curcumin to determine its soluble concentration. To create the standard curve, we had to make two fold dilutions of curcumin in DMSO. Our pooled elutions containing soluble nanoparticles with the highest intensity of yellow coloring were quantified according to the standard curve.
Importantly, we found that the highest loading capacity was when the curcumin telo-disc was mixed prior to adding ApoA1 plasmid during the assembly of the nanoparticle through cell-free production.

In-Vitro curcumin tNLPs can be characterized by SEC



Sonicated for 30 minutes



Sonicated for 30 minutes, and added more curcumin

We also used traditional *in-vitro* approaches for making nanoparticles (**Method 3**). For this reaction we wanted to confirm that the curcumin and the apolipoprotein (ApoA1) co-elute within the same fractions using size exclusion chromatography, which separates large particles from small particles. The particles contain protein that can be measured at 280 nm. In addition, we can obtain a spectral chromatograph of the soluble curcumin at 430 nm. In both sets of graphs we are looking to make sure that peaks are seen at about 2.5 minutes, which is where the expected size range is for properly formed tNLPs. Particles that elute before 2.0 minutes are considered aggregates or void volume.

A.) The first two graphs represent the tNLPs that were made by Method #3, variation #1. Is a variation where curcumin telo-disc were combined with ApoA1 protein and sonicated for 30 minutes before overnight incubation.

B.) The last two graphs represent the tNLPs that were made by Method #3,variation #2. Here, the curcumin telo-disc was combined with ApoA1 protein and sonicated for 30 minutes before overnight incubation. The next morning, more curcumin was added.

Findings and future directions

We managed to create curcumin nano-lipid particles by using three different methods; curcumin telo-disc, cell-free curcumin tNLPs, and *in-vitro* curcumin tNLPs. We showed through both affinity purification and size-exclusion chromatography (SEC) that the homogenous curcumin-tNLPs form with both cell-free and *in-vitro* methods.

The Next Steps:
1. Quantify the amount of curcumin and protein that is in each tNLP.
2. Find the maximum loading capacity of the curcumin-tNLPs.
3. Finally take the tNLPs and test on cell cultures and animal models.

References

Curcumin homing to the nucleolus: mechanism for initiation of an apoptotic program
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4252759/
Curcumin nanodisks: formulation and characterization http://www.sciencedirect.com/science/article/pii/S1549963410002455
http://www.amoebasisters.com/amoebagifs.html
http://www.wikipremed.com/image_archive.php?code=040201
Gupta, Subash C., Sridevi Patchva, and Bharat B. Aggarwal. "Therapeutic Roles of Curcumin: Lessons Learned from Clinical Trials." *The AAPS Journal* 15.1 (2013): 195–218. *PMC*. Web. 21 July 2017.

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