Star shaped PCL-based vehicles for the delivery of HRP in directed enzyme cancer therapy

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TECHNION Israel Institute of Technology

SciTech 2019

Laboratory for Biomaterials

Introduction

Polymer-Directed Enzyme Prodrug Therapy (PDEPT) uses enzymes artificially introduced into the body to convert prodrugs into active drugs in specific tissues [1]. It is a new promising strategy for tumor targeting through enzymatic accumulation at the cancer site, guided by a polymer. PDEPT has great potential to overcome common secondary effects associated with current cancer treatments.

Horseradish Peroxidase (HRP), a naturally found enzyme, can activate a common plant hormone, Indole 3-Acetic Acid (IAA), to form radical species that can damage cancerous cells [2]. Four-armed Polycaprolactone (PCL) is a synthetic, biodegradable and biocompatible polymer that can be used as the delivery matrix. PCL-HRP nanoparticles can be created to be effectively delivered to the tumor site due to the EPR effect and achieve the wanted targeted cytotoxicity (Fig. 1).

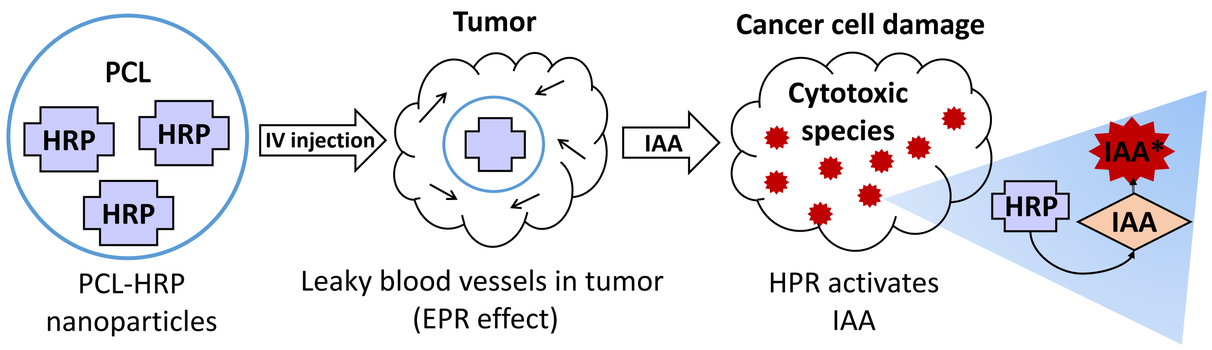


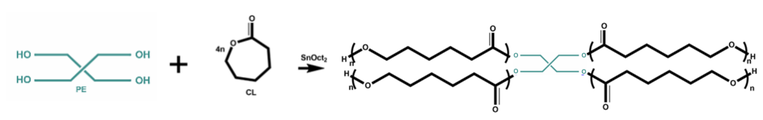
Figure 1. Polymer-Directed Enzyme Prodrug Therapy (PDEPT) mechanism.

Objective

Development of nanometric polymeric vehicles based on Poly (ε-Caprolactone) carrying horseradish peroxidase for their potential use in directed enzyme therapy.

Methods

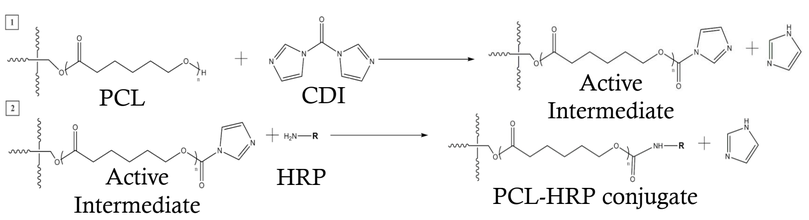
1. Synthesis of star-shaped polycaprolactone polymers



Pentaerythritol + ε-Caprolactone → Four-armed PCL

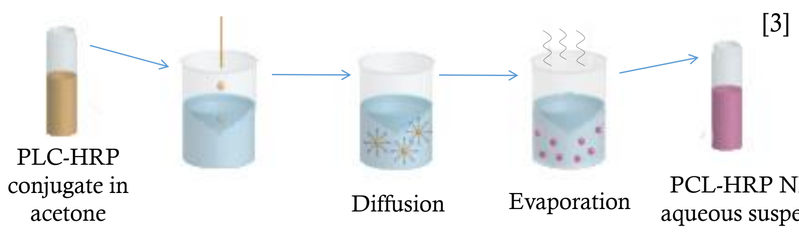
Characterization:
- FT-IR
- GPC

2. PCL activation with CDI and conjugation with HRP simultaneously



Characterization:
- Enzymatic activity

3. Nanoparticle synthesis from PCL-HRP conjugate



[3]

Characterization:
- Enzymatic activity
- DLS
- SEM

Results

PCL characterization

Table 1. PCL molecular weight and polydispersity index measured by GPC

Mn (Da)	24,730
Mw (Da)	30,794
PdI	1.25

The resulting molecular weight for the synthesized PCL approximated the expected value (Table 1). The PdI=1.25 evidenced the homogeneity of the polymeric sample.

The ester carbonyl and aliphatic groups of star-shaped PCL are represented in peaks at 1728 cm^{-1} and 2866 and 2945 cm^{-1}, respectively (Fig. 2).

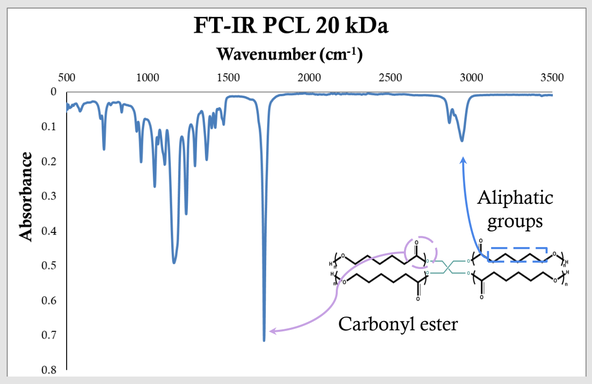


Figure 2. Chemical characterization of PCL 20 kDa polymer using Fourier-Transform Infrared Spectroscopy.

PCL-HRP NPs characterization

Table 2. Enzymatic Activity

	Soluble HRP	PCL-HRP Conjugate	PCL-HRP NPs
IU (µmol/min)	126.17	1.59×10^{-3}	2.47×10^{-3}
$IU/mg_{conjugate}$ (µmol/ min.mg)	_____	1.32×10^{-3}	6.18×10^{-4}

HRP's activity in the conjugate and nanoparticles was lower in comparison to the soluble enzyme (Table 2). The conjugation and nanoprecipitation processes affect the enzymatic activity.

PCL-HRP NPs resulting size was 153.70 nm, with a PdI of 0.179 (Fig. 3).

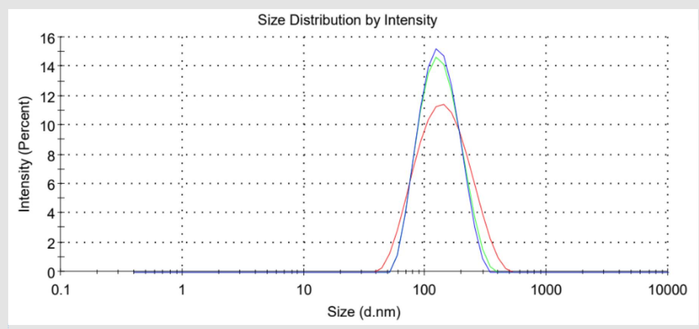


Figure 3. PCL-HRP nanoparticle's size distribution and polydispersity index measured by DLS.

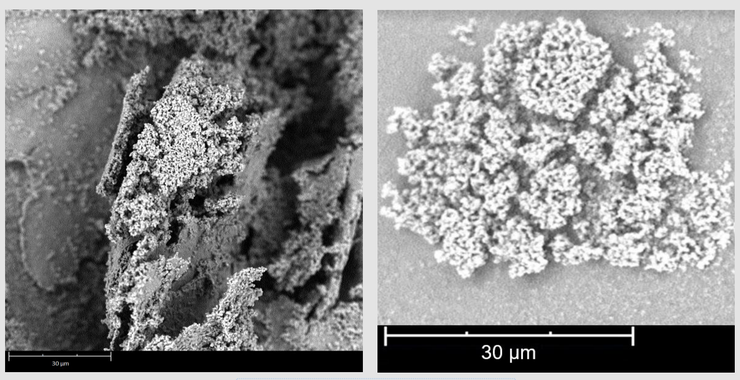


Figure 4. SEM images

Through the Scanning Electron Microscope, it was possible to observe PCL-HRP nanoparticles' shape and organization space (Fig. 4).

Conclusions

Star-shaped PCL 20 kDa polymer was successfully synthesized and conjugated to HRP using the CDI chemistry. PCL-HRP conjugate-based nanoparticles were obtained, showing the desired size and exhibiting HRP's enzymatic activity. These nanometric structures have great potential for the development of a PDEPT strategy as a novel cancer therapy.

Acknowledgements

We would like to thank MSc. Student Veronica Moskovicz and Assoc. Prof. Boaz Mizrahi for hosting and guiding us through our research in his laboratory. We would also like to thank the foundations and donors for their generous support of the SciTech Program.

References

[1] - Xu G, Mcleod HL. Strategies for enzyme/prodrug cancer therapy. American association for cancer research. 2001;7:3314.
[2] - Azevedo AM, et al. Horseradish peroxidase: a valuable tool in biotechnology. Biotechnology annual review. 2003;9:199.
[3] - Nicolas J, et al. Design, functionalization strategies and biomedical applications of targeted biodegradable/biocompatible polymer-based nanocarriers for drug delivery. Chemical society reviews. 2013;42(3):1147.