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Lab & Production Materials

Protocol

NanoFabTx™ NanoFlash PEG-PCL drug formulation screening kit

For CIJ synthesis of nanoparticles

Protocol for Catalog No # 933090

Introduction

This kit is designed for encapsulation of hydrophobic drugs in specifically sized, biodegradable, PEGylated PCL nanoparticles for drug delivery research applications. The kit contains rationally selected PEGylated PCL polymers that have been widely used in drug delivery systems for controlled drug release of many different types of therapeutic molecules. PEGylated polymers used for synthesis result in nanoparticles with a PEG surface coating that reduces protein absorption and prolongs circulation time, leading to slow and sustained release of the encapsulated drug. Drug encapsulated particles synthesized with this kit are suitable for biomedical research applications such as oncology, immuno-oncology, gene delivery, and vaccine delivery. This kit provides reagents and protocols for flash nanoprecipitation method using a Confined Impinging Jet (CIJ) to synthesize nanoparticles ranging from 50 nm to 150 nm in size. This allows users to select the optimal polymer and concentration to achieve the desired drug loading and nanoparticle size. The kit also includes protocols for both hand-operated and syringe pump-operated CIJ-based synthesis and is used with the **NanoFabTx™ NanoFlash CIJ instrument**, including the CIJ device, fittings, and tubing required to get started with the CIJ-based synthesis process.

Disclaimer

NanoFabTx™ NanoFlash PEG-PCL drug formulation screening kit is for research use only; not suitable for human use. Please consult the Safety Data Sheet for information regarding hazards and safe handling particles.

Storage and stability: Store kit at 2-8°C. Protect from light. Refer to the expiration date on the batch-specific Certificate of Analysis.

Specifications

Storage	Store TissueFab™ - HAMA-UV bioink at 2 - 8 °C. Protect from light by storing the bottle in a foil bag or wrapping it in aluminum foil.
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials

Materials supplied

Each NanoFabTx™ NanoFlash PEG-PCL drug formulation screening kit is supplied with the following:

MATERIAL	QUANTITY
PEGPCL-L	500mg
PEGPCL-M	500mg
PEGPCL-H	500mg
Stabilizer-P	5g

Materials required, but not supplied

Catalog Number	Quantity
401757	Tetrahydrofuran (THF) or solvent of choice
Z693472	Magnetic Stirrer
Z266337	Stir bars (40 mm x 8 mm)
27024	Glass vials, clear glass (4 ml capacity)
27172-U	Glass vials, clear glass (22 ml capacity)
SLFH025	Syringe filters 0.45µm (for filtering non-aqueous solutions like polymer/drug solutions)
SLHAR33SS	Syringe filters 0.45µm (for filtering aqueous solutions like stabilizer solution)
D9777	Dialysis membrane (12-14kDa cut off)
	Deionized water
01885	Docetaxel or hydrophobic drug of choice
	Syringes compatible with syringe pumps required (recommended with Hamilton® GASTIGHT® syringes)
	Syringes compatible with syringe pumps required (recommended with Hamilton® GASTIGHT® syringes)

Materials required for syringe pump method, but not supplied:

Item description
Syringe pump (protocol requires one pump)-recommended with Harvard Apparatus – PHD Ultra pumps

Before you start: Important tips for optimal bioprinting results

Polymer and drug solution preparation:

- Prepare the desired formulation provided in table 1 (e.g., add 10 mg of one of the three polymers included in the polymer kit and 10 mg of the drug to 5 ml of THF to prepare a 0.2 wt/v% of polymer and drug solution). Organic solvents such as ACN, DMSO, and THF are recommended as they have a high solubility for both the drug and polymer.

Note: *Concentrations provided in table 1 are optimized for the model drug, docetaxel. Utilizing a different drug molecule and alternate concentrations may result in variations in nanoparticle properties, including size and encapsulation efficiency.*

- Gently vortex the solution for 1-2 minutes to fully dissolve the polymer and drug. The final solution should be a clear and transparent mixture.

Note: *If necessary, heating the polymer solution can aid in dissolving the polymers.*

- Before use, filter the solution through a 0.45 µm syringe filter to remove any particulate matter. Please note that the polymer solution should not be stored for longer than 24 hours.

Stabilizer solution preparation:

- Prepare a 0.1 wt/v% of stabilizer in deionized water (e.g., add 0.05 g of the stabilizer to 50 ml deionized water to prepare a 0.1 wt/v% of the stabilizer solution).
- Place the solution on a heated magnetic stir plate and stir the solution gently. While stirring, warm the solution to 50–60 °C to completely dissolve the stabilizer. The final solution should be a clear transparent solution. You may also use a heat gun to warm the solution.
- Let the stabilizer solution cool down to room temperature and filter it through a 0.45 µm syringe filter before use.

Procedure

Procedure 1- Hand-operation with subsequent dilution to synthesize drug-encapsulated PEG-PCL nanoparticles:

CIJ instrument setup: Follow the instructions provided for the NanoFabTx™ NanoFlash CIJ instrument. If using with a different CIJ instrument, additional optimization of the following protocol may be required.

Prime the CIJ instrument:

- Fill two syringes with THF or solvent of interest and load them on CIJ.
- Use the metal plate to connect two syringes and push the syringes rapidly
- Collect the solvent at the outlet in a waste container and repeat the process three times.

Note: *Priming purges gases from the fluid pathways and serves as a check of chemical compatibility for all wetted parts of the system. In addition, priming reduces or prevents precipitation of polymers and drug inside the system. Precipitation of polymers and drug can obstruct the mixer channels and tubing.*

Drug-loaded PEG-PCL nanoparticle synthesis:

- Once the polymer and drug solution has been prepared, transfer the desired volume of the solution into a syringe.
- In a separate syringe, transfer an equal volume of the prepared stabilizer solution.
- Attach both syringes containing the polymer + drug solution and stabilizer solution onto the CIJ device.
- Transfer 8 times the volume of polymer and drug solution of the stabilizer solution into a beaker.
- Use the metal plate to connect two syringes to ensure simultaneous actuation and push the syringes in a rapid motion.
- Collect the nanosuspension at the outlet tube into the beaker of stabilizer solution. Note: Discard the first and last drops of the outlet suspension to obtain a more monodispersed particle population.

Clean the CIJ instrument:

- Clean the CIJ instrument by filling two syringes with THF or your desired solvent and loading them onto the CIJ.
- Connect the two syringes with the metal plate and rapidly push them to ensure simultaneous actuation.
- Collect the solvent at the outlet into a waste container and repeat the process three times.
- Repeat the previous steps with DI water and store the device.
  Note: Improper cleaning can result in blockage in the mixer channels and tubing.

Remove excess stabilizer, solvent, and non-encapsulated drug:

- Transfer the drug-encapsulated nanoparticle suspension to a 12-14kDa cut-off cellulose membrane.
- Dialyze against 4L deionized water for 4-6 h at room temperature, change the water after 30 minutes and 1 hour.
- Transfer the dialyzed drug-encapsulated nanoparticles solution to a glass vial and store at 2-4°C for further analysis.

Procedure 2- Syringe pump-operation with subsequent dilution to synthesize drug-encapsulated PEG-PCL nanoparticles:

CIJ instrument setup: Follow the instructions provided for the NanoFabTx™ NanoFlash CIJ instrument. If using with a different CIJ instrument, additional optimization of the following protocol may be required.

Prime the CIJ instrument:

- Fill two syringes with THF or solvent of interest and load the syringes on the syringe pump.
- Adjust the flow rate at 30 ml/min.
- Run the syringe pump and collect the solvent at the outlet in a waste container and repeat the process three times.
  Note: Priming purges gases from the fluid pathways and serves as a check of chemical compatibility for all wetted parts of the system. In addition, priming reduces or prevents precipitation of polymers and drug inside the system. Precipitation of polymers and drug can block the mixer channels and tubing.

Drug-loaded PEG-PCL nanoparticle synthesis:

- Once the polymer and drug solution has been prepared, transfer the desired volume of the solution into a syringe.
- In a separate syringe, transfer an equal volume of the prepared stabilizer solution.
- Attach both syringes containing the polymer + drug solution and stabilizer solution onto the CIJ device.
- Transfer 8 times the volume of polymer and drug solution of the stabilizer solution into a beaker.
- Load the syringes on the syringe pump.
- Adjust the flow rate to 30 ml/min

  Note: *The particle size can be adjusted by varying the flow rate and concentration of the polymer and drug. Typically, a decrease in both the concentration of the polymer and drug and the flow rate results in the formation of smaller nanoparticles.*

- Run the syringe pump and collect the nanosuspension at the outlet into the beaker of stabilizer solution.

  Note: *To obtain more monodispersed particles, spare the first and last drops of the outlet suspension.*

Clean the *CIJ instrument*:

- Clean the CIJ instrument by filling two syringes with THF or your desired solvent and loading them onto the CIJ.
- Adjust the flow rate at 30 ml/min.
- Run the syringe pump and collect the solvent at the outlet in a waste container and repeat the process three times.
- Repeat the previous steps with DI water and store the device.

  Note: *Improper cleaning can result in blockage in the mixer channels and tubing.*

Table 1. List of formulation suggested to be prepared

Formulation	Initial polymer concentration (wt/v %)	Initial drug concentration (wt/v %)	Final polymer concentration (wt/v %)	Final drug concentration (wt/v %)
0.2 wt% polymer - no drug	0.2	0	0.02	0
0.2 wt% polymer - 0.1 wt% drug	0.2	0.1	0.02	0.01
0.2 wt% polymer - 0.2 wt% drug	0.2	0.2	0.02	0.02
0.5 wt% polymer - no drug	0.5	0	0.05	0
0.5 wt% polymer - 0.25 wt% drug	0.5	0.25	0.05	0.025
0.5 wt% polymer - 0.5 wt% drug	0.5	0.5	0.05	0.05
1 wt% polymer - 0wt% drug	1	0	0.1	0
1 wt% polymer – 0.5 wt% drug	1	0.5	0.1	0.05
1 wt% polymer - 1 wt% drug	1	1	0.1	0.1

Physiochemical characterization of nanoparticles:

Nanoparticles are usually characterized in terms of size, morphology, zeta potential, drug content and cytotoxicity. A wide range of techniques are available for the physiochemical characterization of nanoparticles and some of the most used techniques are listed in table 2.

Table 2. Principle techniques for physiochemical characterization of nanoparticle

Parameter	Technique
Size and morphology	Dynamic light scattering Transmission electron microscopy Scanning (electron, force) microscope
Zeta potential	Dynamic light scattering
Drug content	High-performance liquid chromatography Ultraviolet-visible spectroscopy
Cell uptake and cytotoxicity	LDL uptake assay MTT cytotoxicity assay LDH cytotoxicity assay

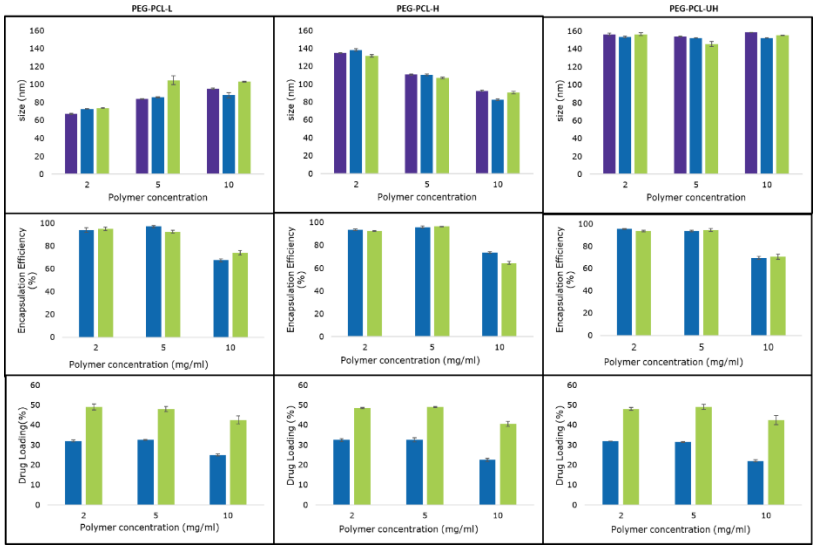

Figure 1. Example data of nanoparticle size, encapsulation efficiency and drug loading versus polymer concentration and polymer identity using a model small molecule drug. No drug (purple), 1:2 drug:polymer ratio (blue), 1:1 drug:polymer ratio (green). PDI <0.2.