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Scriptwriter Name: Bridget Colvin

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Title: Synthesis of Aptamer-PEI-g-PEG Modified Gold Nanoparticles Loaded with Doxorubicin for Targeted Drug Delivery

Authors and Affiliations: Lei Nie^{1,2}, Shaolan Sun¹, Meng Sun¹, Qiuju Zhou³, Zongwei Zhang³, Lingyun Zheng³, and Lingling Wang³

¹College of Life Sciences, Xinyang Normal University

²Department of Imaging & Pathology, University of Leuven and Oral & Maxillofacial Surgery, University Hospitals Leuven

³Analysis & Testing Center, Xinyang Normal University

Corresponding Author:

Lei Nie

nieleifu@yahoo.com

nielei@xynu.edu.cn

Co-authors:

sunshaolan@foxmail.com

sunmeng2010abc@163.com

zhouqiuju@iccas.ac.cn

zongwen500@sina.com

zhengly@xynu.edu.cn

wfulful@163.com

Author Questionnaire

1. **Microscopy:** Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**
2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **N**
3. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. **Lei Nie/Shaoan Sun**: Because of drug resistance and toxicity, the use of doxorubicin is limited in the clinic. Our protocol provides a degradable carrier for controlling the release of this chemotherapeutic agent **[1]**.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

- 1.2. **Lei Nie/Shaoan Sun**: The advantage of this method is that the synthesized carrier can be used to deliver the drug directly to cancer cells, inducing cell death in the cancer cells only **[1]**.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Double-Carboxyl Polyethylene Glycol (CT-PEG) Synthesis

- 2.1. For CT-PEG (C-T-peg) synthesis, first add 1.46 grams of succinic anhydride and 209 milligrams of 4-dimethylaminopyridine to a 100-milliliter, round bottom flask [1].
 - 2.1.1. WIDE: Talent adding SA and/or DAMP to flask, with SA and DAMP containers visible in frame
- 2.2. Next, add 15 milliliters of anhydrous tetrahydrofuran to the flask [1] and fit a glass stopper into the flask [2] before incubating the reaction for 30 minutes at 0 degrees Celsius [3].
 - 2.2.1. Talent adding THF to flask, with THF container visible in frame
 - 2.2.2. Talent adding stopper to flask
 - 2.2.3. Talent placing flask at 0 °C
- 2.3. During the incubation, add 4.2.8 grams of PEG, 1.8 milliliters of triethylamine, and 15 milliliters of anhydrous tetrahydrofuran to a new flask [1] and close the flask with a glass stopper [2].
 - 2.3.1. Talent adding PEG and/or TEA and/or THF to flask, with PEG, TEA, and THF containers visible in frame *Videographer: Important step*
 - 2.3.2. Talent placing stopper *Videographer: Important step*
- 2.4. At the end of the incubation, use a syringe under nitrogen atmosphere to slowly transfer this second solution to the first flask [1].
 - 2.4.1. Solution being transferred
- 2.5. Stir the new solution for 2 hours at 0 degrees Celsius [1] before continuing the reaction at room temperature overnight [2].
 - 2.5.1. Solution being stirred at 0 °C
 - 2.5.2. Talent placing solution at RT
- 2.6. The next morning, use a rotary evaporator at 40 degrees Celsius and 0.1 megapascals to concentrate the reaction solution and to remove the tetrahydrofuran solvent [1].
 - 2.6.1. Shot of flask in evaporator

2.7. After an hour, dissolve the reaction solution in 15 milliliters of 1.325 gram/milliliter of dichloromethane at room temperature [1] before adding 15 milliliters of cold diethyl ether to obtain the PEG diacid precipitation product [2-TXT].

2.7.1. Talent adding DCM to flask, with DCM container visible in frame *Videographer: Important/difficult step*

2.7.2. Talent adding cold diethyl ether to flask, with diethyl ether container visible in frame *Videographer: Important/difficult step* TEXT: **Optional: Repeat precipitation x3**

2.8. Then remove the solvent via filter paper [1] and dry the precipitate under vacuum for 48 hours at room temperature [2].

2.8.1. Shot of precipitate, then solution being filtered

2.8.2. Talent placing precipitate under vacuum

3. Poly(ethylenimine) Grafted with Polyethylene Glycol (PEI-g-PEG) Copolymer Synthesis

3.1. For PEI-g-PEG (P-E-I-G-PEG) copolymer synthesis, add 305.47 milligrams of the CT-PEG precipitate and 5 milliliters of DMSO (D-M-S-O) to a new flask [1-TXT] and stir the reaction at room temperature until the CT-PEG is fully dissolved [2].

3.1.1. WIDE: Talent adding precipitate and/or DMSO to flask, with DMSO container visible in frame TEXT: **DMSO: dimethyl sulfoxide**

3.1.2. Reaction being stirred

3.2. Next, dissolve 49.46 milligrams of EDC (E-D-C) in 5 milliliters of DMSO [1-TXT] and add the solution to the flask [2].

3.2.1. Talent adding EDC to DMSO, with EDC and DMSO containers visible in frame TEXT: **EDC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride**

3.2.2. Talent adding solution to flask

3.3. Stir the reaction for 30 minutes at room temperature [1] before dissolving 29.69 milligrams of N-hydroxysuccinimide in 5 milliliters of DMSO [2] and adding the resulting solution to the flask [3].

3.3.1. Reaction being stirred

3.3.2. Talent adding NHS to DMSO, with NHS and DMSO containers visible in frame

3.3.3. Talent adding solution to flask

3.4. Continue to stir the reaction for 3 hours at room temperature [1].

3.4.1. Reaction being stirred

3.5. Near the end of the incubation, dissolve 28.6 microliters of PEI into 10 milliliters of DMSO [1] and add the solution dropwise to the flask [2].

3.5.1. Talent mixing PEI in DMSO, with PEI and DMSO containers visible in frame

Videographer: Important step

3.5.2. Talent adding drops of solution to flask *Videographer: Important step*

3.6. Stir the reaction at room temperature for at least 3 days [1].

3.6.1. Reaction being stirred

3.7. At the end of the incubation, transfer the reacted solution to a dialysis bag with a 1000-molecular weight cut-off [1] and place the bag into a 1-liter beaker containing 500 milliliters of ultrapure water as the dialysate [2-TXT].

3.7.1. Talent adding solution to bag

3.7.2. Talent placing bag into beaker **TEXT: Change ultrapure H₂O every 12 h for 3 d**

3.8. At the end of the dialysis, transfer the solution to a dialysis bag with a 10,000-molecular weight cut-off [1] and place the bag into a 1-liter beaker with 500 milliliters of ultrapure water as the dialysate [2-TXT].

3.8.1. Talent adding solution to bag

3.8.2. Talent placing bag into beaker **TEXT: Change ultrapure H₂O every 12 h for 3 d**

3.9. At the end of the second dialysis, use a rotary evaporator at 40 degrees Celsius and 0.1 megapascals to concentrate the solution [1] and freeze-dry the sample to obtain the PEI-g-PEG powder [2].

3.9.1. Talent placing flask into evaporator

3.9.2. Talent freeze-drying sample OR Shot of freeze-dried sample

4. PEI-g-PEG Coated Gold Nanoparticles (@AuNPs) Synthesis

4.1. For the coating of gold nanoparticles with the PEI-g-PEG product, dissolve 5 milligrams of the prepared PEI-g-PEG in 5 milliliters of ultrapure water in a new flask [1] and fit the flask with a glass stopper [2].

4.1.1. WIDE: Talent adding PEI-g-PEG to flask

4.1.2. Talent placing stopper into flask

4.2. Add 100 milliliters of 0.3-millimolar auric chloride solution to the flask [1] and stir the solution for 3 hours at room temperature. The color should change immediately from light yellow to dark yellow [2].

4.2.1. Talent adding solution to flask, with HAuCl_4 container visible in frame

4.2.2. Solution being stirred/changing color

4.3. At the end of the incubation, add 1 milliliter of 1 milligram/milliliter of sodium borohydride solution to the flask, the color should change immediately from dark yellow to golden yellow [1] and stir the solution for an additional 3 hours at room temperature [2].

4.3.1. Talent adding solution to flask, with NaBH_4 container visible in frame

Videographer: Important step

4.3.2. Solution being stirred *Videographer: Important step*

4.4. Then dialyze the reaction product with 1000 molecular weight cutoff dialysis bag for 3 days as demonstrated [1] to obtain the PEI-g-PEG-coated gold nanoparticle solution [2].

4.4.1. Talent adding solution to bag

4.4.2. Shot of solution

5. DOX-g-PEI-g-PEG@AuNP Synthesis

5.1. For grafting of doxorubicin to the PEI-g-PEG-coated gold nanoparticles, add 1 milliliter of 2.2 milligram/milliliter doxorubicin solution and 20 milliliters of the PEI-g-PEG coated gold nanoparticle solution to a new flask [1] and fit the flask with a glass stopper [2].

5.1.1. WIDE: Talent adding DOX and/or PEI-g-PEG@AuNP to flask, with both solution containers visible in frame

5.1.2. Talent placing stopper into flask

5.2. Next, dissolve 0.727 milligrams of EDC in 1 milliliter of ultrapure water [1] and add the EDC solution to the flask [2].

5.2.1. Talent mixing EDC with water, with EDC container visible in frame

5.2.2. Talent adding solution to flask

- 5.3. Dissolve 0.437 milligrams of N-hydroxysuccinimide in 1 milliliter of ultrapure water [1] and add the N-hydroxysuccinimide solution to the flask for a 1-hour incubation with stirring at room temperature [2].

- 5.3.1. Talent dissolving NHS in water, with NHS container visible in frame

- 5.3.2. Talent adding solution to flask

- 5.4. At the end of the incubation, dialyze the reaction product in a 1000-molecular weight cutoff dialysis bag for 3 days as demonstrated [1] to obtain the doxorubicin-g-PEI-g-PEG-coated gold nanoparticle solution [2].

- 5.4.1. Talent adding solution to bag

- 5.4.2. Shot of solution

6. AS1411-g-DOX-g-PEI-g-PEG@AuNP Synthesis

- 6.1. For grafting of the AS1411 (A-S-one-four-eleven) aptamer to the DOX-g-PEI-g-PEG, add 20 milliliters of the doxorubicin-g-PEI-g-PEG coated gold nanoparticle solution to an approximately 4 optical density concentration of AS1411 [1-TXT].

- 6.1.1. WIDE: Talent adding solution for AS1411 **TEXT: 1 OD = approximately 33 micrograms**

- 6.2. Dissolve 28.76 milligrams of EDC in 1 milliliter of ultrapure water [1] and add the EDC solution to the flask [2].

- 6.2.1. Talent mixing EDC with water, with EDC container visible in frame

- 6.2.2. Talent adding EDC solution to flask

- 6.3. Dissolve 17.27 milligrams of N-hydroxysuccinimide in 1 milliliter of ultrapure water [1] and add the N-hydroxysuccinimide solution to the flask for a 1-hour incubation with stirring at room temperature [2].

- 6.3.1. Talent dissolving NHS in water, with NHS container visible in frame

- 6.3.2. Talent adding solution to flask

- 6.4. Then dialyze the reaction product in a 1000-molecular weight cutoff dialysis bag for 3 days as demonstrated [1] to obtain AS1411-g-doxorubicin-g-PEI-g-PEG-coated gold nanoparticles [2].

- 6.4.1. Talent adding solution to bag

- 6.4.2. Shot of AS1411-g-doxorubicin-g-PEI-g-PEG@AuNP

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.3., 2.7., 3.5., 4.3.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
2.7.

Results

7. Results: Representative AuNP Characterization

- 7.1. Proton NMR (**N-M-R**) spectroscopy [**1-TXT**] can be used to confirm the successful synthesis of CT-PEG polymer [**2**] and PEI-g-PEG copolymers [**3**].
 - 7.1.1. LAB MEDIA: Figure 2 **TEXT: NMR: nuclear magnetic resonance**
 - 7.1.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize spikes at 3.61 and 2.57*
 - 7.1.3. LAB MEDIA: Figure 2 *Video Editor: please emphasize spikes at 2.6 and 1.66*
- 7.2. Ultra-violet spectroscopy can be conducted to determine the successful functionalization and gradual loading of prepared copolymer on gold nanoparticles [**1**].
 - 7.2.1. LAB MEDIA: Figure 3B *Video Editor: please sequentially add/emphasize numbers and arrows to peaks*
- 7.3. X-ray photoelectron spectroscopy can be used to investigate the chemical bond of copolymer on gold nanoparticles [**1**].
 - 7.3.1. LAB MEDIA: Figure 4A *Video Editor: please sequentially add/emphasize right graphs from C1s to Au4f*
- 7.4. Dynamic light scattering can be performed to determine the size distribution of the prepared nanoparticles [**1**].
 - 7.4.1. LAB MEDIA: Figure 5 *Video Editor: please sequentially emphasize red, black, and blue data lines*
- 7.5. In this analysis, transmission electron microscopy revealed non-clustered nanoparticles with a uniform morphology [**1**].
 - 7.5.1. LAB MEDIA: Figure 6
- 7.6. Cell viability analysis revealed a decrease in A549 (**A-five-four-nine**) cell numbers over time [**1**] and in response to increasing concentrations of nanoparticle [**2**].
 - 7.6.1. LAB MEDIA: Figures 7 and Figure 8 *Video Editor: please emphasize Free Dox and AuNP 48 h data bars and Figures 8B and 8D*

- 7.6.2. LAB MEDIA: Figures 7 and 8 *Video Editor: please stretch arrow from without adding nanoparticles to 220 microgram/mL data bars to indicate decrease and Figure 8B*
- 7.7. Compared to the free doxorubicin group **[1]**, the cell numbers increased, however, indicating a reduced toxicity **[2]**.
- 7.7.1. LAB MEDIA: Figure 7 *Video Editor: please emphasize Free Dox data bars*
- 7.7.2. LAB MEDIA: Figure 7 *Video Editor: please stretch arrow from 220 microgram/mL data bars to without adding nanoparticles to indicate increase*
- 7.8. Further, analysis of the doxorubicin release profile **[1]** reveals that the sustained release of doxorubicin from functionalized nanoparticles caused a decrease in A549 cells **[2]**.
- 7.8.1. LAB MEDIA: Figure 9
- 7.8.2. LAB MEDIA: Figure 9 *Video Editor: please emphasize data line*

Conclusion

8. Conclusion Interview Statements

- 8.1. **Lei Nie/Shaoan Sun**: The PEI-g-PEG copolymer preparation and AS1411 grafting steps are important for a successful implementation of the procedure [1].
 - 8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.5, 6.1)
- 8.2. **Lei Nie/Shaoan Sun**: The same drug carrier can be achieved by grafting AS1411 before grafting DOX, but the drug loading efficiency will be decreased [1].
 - 8.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera