Phenylboronic acid functionalized poly (lactic-co-glycolic acid) nanoparticles towards lung cancer

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BACKGROUND

Introduction: Lung cancer is the leading cause of death among all types of cancer, with a death severity of 350 deaths per day in the United States [1]. The multiple anti-cancer activities of Biochanin A (BCA) and Lenvatinib (LTB) make an ideal chemo-herbal combination that can synergize with each other [2]. The toxicity towards normal cells is the biggest obstacle during cancer therapies. Targeted drug delivery (TDD) identifies such challenges by employing a suitable ligand to achieve site-specificity, safety, and deliverability [3].

Challenges: Development of drug resistance, systemic toxicities, and treatment variability among patients limit the translational outcomes of chemotherapeutics [4].

Research Approach: Lung cancer patients frequently overexpress the sialic acid epitopes, which provide binding affinity and precise therapy for phenyl boronic acid (PBA) functionalized nanocarriers.
Aim: **a.** To synthesize and characterize the PBA-PLGA; **b.** To develop and evaluate the in-vitro and in-vivo performance of PBA-PLGA-BCA-LTB NPs

Methodology

Synthesis of PBA-PLGA

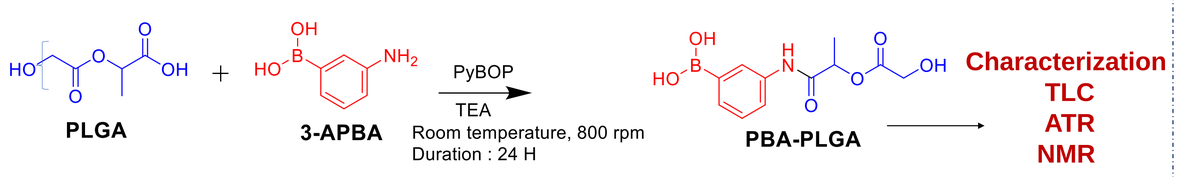


Figure 1: Process of synthesis of PBA-PLGA

Development of dual drug loaded PBA-PLGA NPs

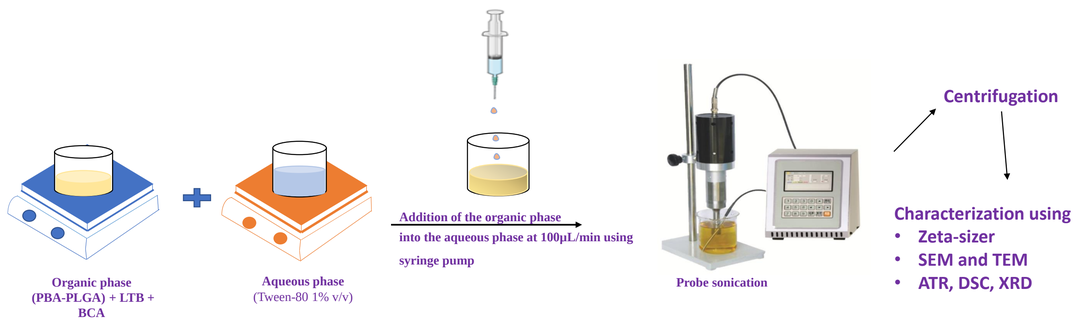
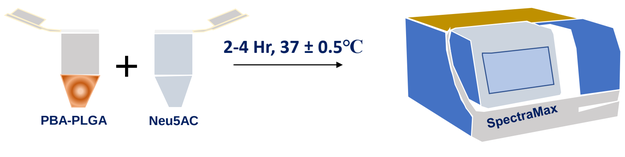


Figure 2 : Method of preparation of PBA-PLGA-BCA-LTB NPs

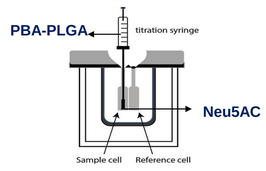
In-vitro studies

I. In-vitro drug release

Ⅱ. In-vitro binding affinity of PBA-PLGA to N-acetyl Neuraminic acid or sialic acid

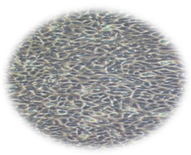


a. Fluorescent quenching assay



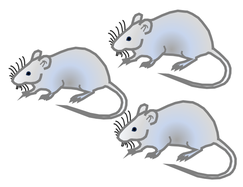
b. Isothermal titration calorimetry (ITC)

III. Cellular studies in A549 cells



→ Cytotoxicity
→ Cellular uptake (Qualitative and Quantitative)
→ Cell migration assay

In-vivo studies



Intravenous administration → Blood collection - ROP

1. **Pharmacokinetic studies**
2. **Biocompatibility studies**
   a. Total blood count
   b. Biochemical evaluation
   c. Histological evaluation

IAEC Approval No- NIPER/PE/2024/56

Results

Characterization of PBA-PLGA

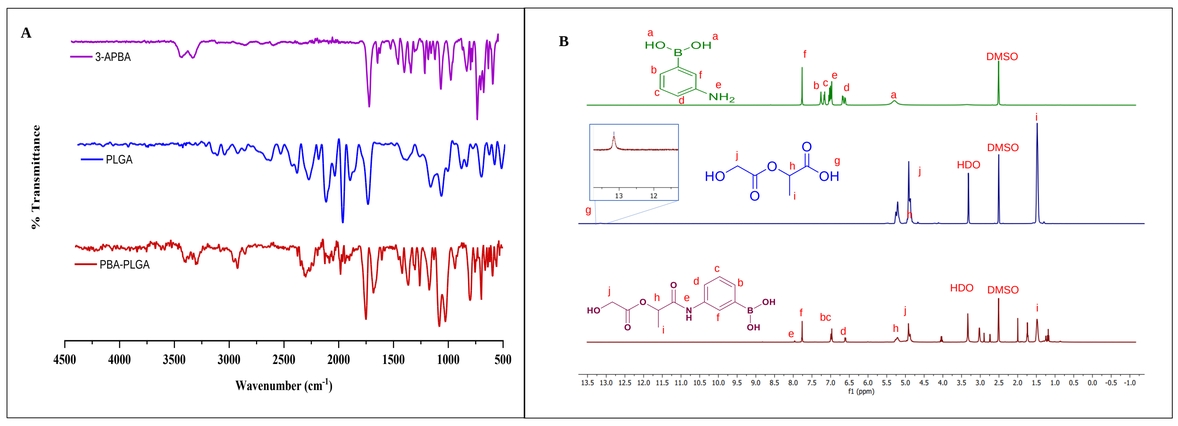


Figure 3: **A.** ATR spectroscopy and**; B.** Overlay NMR spectra of 3-APBA (upper), PLGA (middle) and synthesized PBA-PLGA (lower).

Quality by design-driven development of PBA-PLGA BCA-LTB NPs

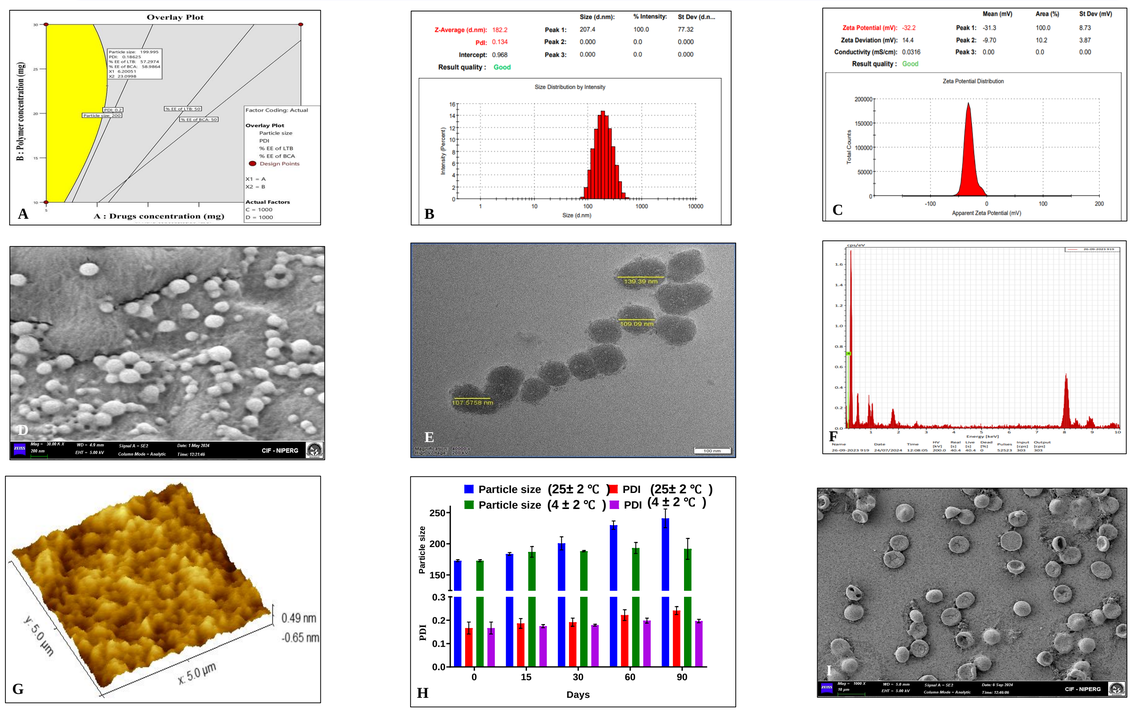


Figure 4:Development of PBA-PLGA-BCA-LTB NPs **A.** Optimized design space using Design Expert; **B.** Particle size and PdI; **C.** Zeta potential; **D.** SEM**; E.** TEM image; **F.** EDX spectrum representing the presence of boron; **G.** Atomic force microscopic image illustrating 3D view of topography; **H.** Storage stability study explaining change in particle size and PDI after 3 months; **I.** SEM image of RBCs after treatment with 320µg/mL concentration of PBA-PLGA-BCA-LTB NPs.

In-vitro studies

Ⅰ. In-vitro drug release

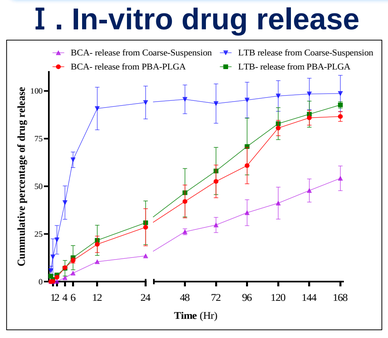


Figure 5: In-vitro BCA and LTB release from PBA-PLGA-BCA-LTB NPs and Coarse-BCA-LTB.

Ⅱ. In-vitro binding affinity studies

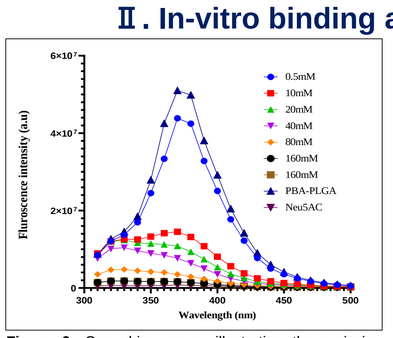


Figure 6: Quenching assay illustrating the emission spectra of PBA-PLGA (100 µM) when quenched with 0.5mM, 10mM, 20mM, 40mM, 80, 160mM and 320mM concentrations of Neu5AC.

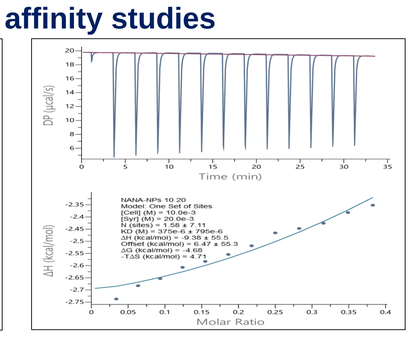


Figure 7: Isothermal titration calorimetry binding study of (Neu5AC) depicting the binding curve between Neu5AC and PBA-PLGA.

Ⅲ. Cellular studies in A549 cells

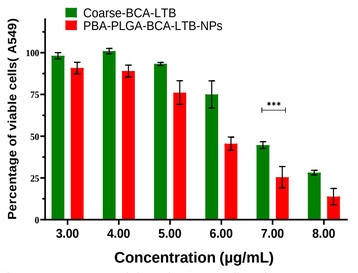


Figure 8 : Cytotoxicity of A549 cells after treatment with different concentrations of BCA-LTB-coarse suspension and PBA-PLGA-BCA-LTB NPs. Data was calculated using one-way analysis of variance followed by Tukey's multiple comparison test, whereas *** represents p-value < 0.001

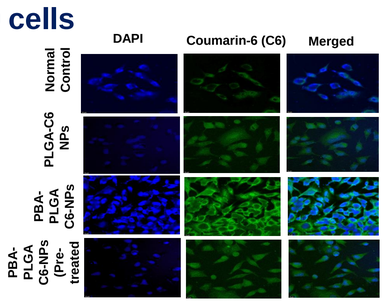


Figure 9 : Confocal images of A549 cells after treatment of Free-C6 (control), C-6 loaded PBA-PLGA, C-6 loaded PLGA NPs, and C-6 loaded PBA-PLGA after PBA saturation for 4 hrs, at 5µg/mL concentration for 6 hrs.

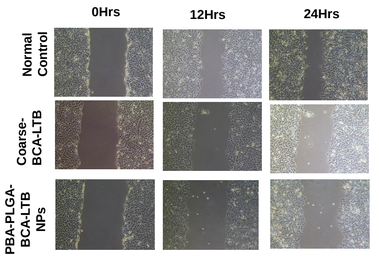


Figure 10 : Microscopic images illustrating the percentage of A549 cell migration after treatment with Control, Coarse-BCA-LTB, and PBA-PLGA-BCA-LTB NPs for 0, 12, and 24 hrs.. (Scale used 10um)

In-vivo studies

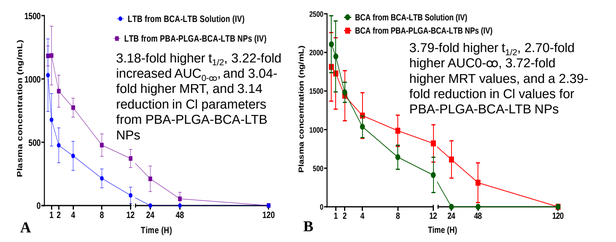


Figure 11 : In-vivo pharmacokinetic profile of **(A)** LTB (4mg/kg) **(B)** BCA (20mg/kg) in Wistar rats after intravenous administration from free BCA-LTB solution and PBA-PLGA-BCA-LTB-NPs.

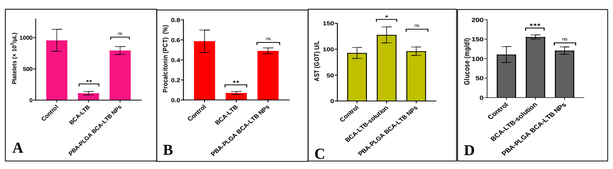


Figure 12 : Hematological profile and biochemical levels after treatment with normal saline, BCA-LTB-solution, PBA-PLGA-BCA-LTB NPs depicting the concentration of **A.** Platelets, **B.** Procalcitonin, **C.** Aspartate aminotransferase (AST)/ and **D.** Glucose; The significant differences between BCA-LTB solution, and PBA-PLGA-BCA-LTB NPs with a control group were calculated using one-way analysis of variance followed by Tukey's multiple comparison test, whereas *** represents p-value < 0.001,** p < 0.005, ns- non-significant p >0.05.

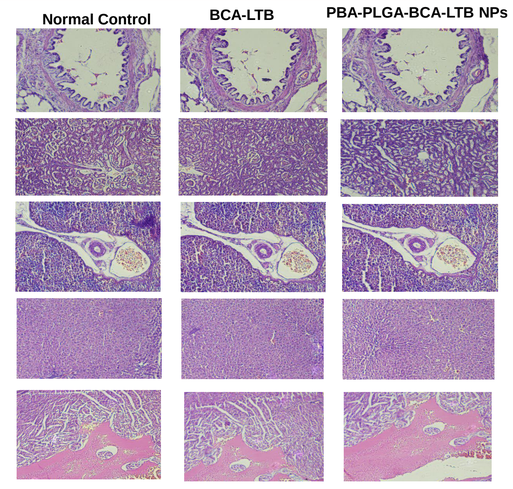


Figure 13 : Hematoxylin and Eosin (H&E) stained images of different organs after treatment with normal saline, BCA-LTB-solution, PBA-PLGA-BCA-LTB NPs for 28 days. (Images were collected at 20x magnification).

Conclusion

PBA-PLGA is a biocompatible, non-toxic biomaterial that can be used to achieve sialic acid-mediated cancer targetability. It is suitable for co-loading two different drugs and achieving sustained drug characteristics to produce a longer therapeutic effect. Future studies are required to evaluate the anti-cancer potential of PBA-PLGA-BCA-LTB NPs using suitable in-vivo lung cancer models.

References

[1] Siegel et al, Cancer J Clin, 2022:7-33, [2] Youssef et al, Sci. Rep, 2016:30717.
[3] Lee et al, Adv. Funct. Mater,2015:3705-3717, [4] Elgohary et al, JCR, 2018:230-243.
[5] Kumar et al, Nanoscale, 2025,17, 15960-15987

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