Quick Isolation Of Small Exosome From Human Serum By Their Mass Enhancement Using Gold Nanoparticles

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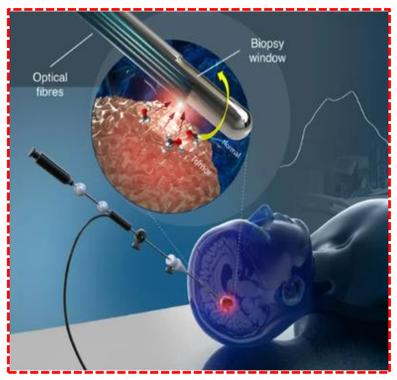
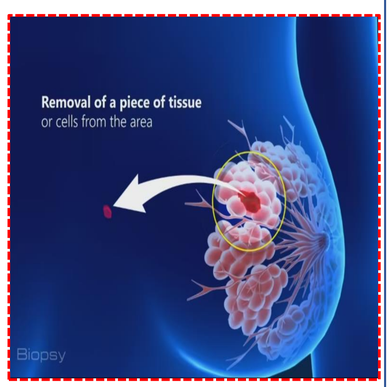
INTRODUCTION

Motivation

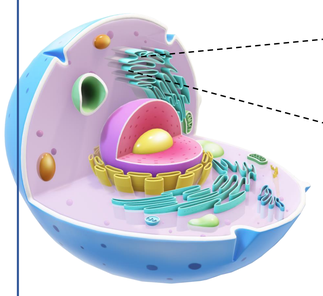
- Cancer is the top cause of death in various countries. In 2040, cancer cases are anticipated to rise 47% from 2020[1].
- Delay in cancer therapy is a major cause of increased cancer deaths[2,3].Infrastructure improvement is limited. Literature demonstrates that delaying cancer therapy by even four weeks can increase mortality.[4].
- In tissue biopsy, the needle used to remove tumor tissue can seed tumor cells along its path, causing difficulties and tumor spread.[5].
- Cell search is the only FDA-approved method for estimating Circulating Tumor Cells (CTC) in blood, but its clinical benefits are unproven[6].Developing cost-effective technologies for early cancer detection is vital.

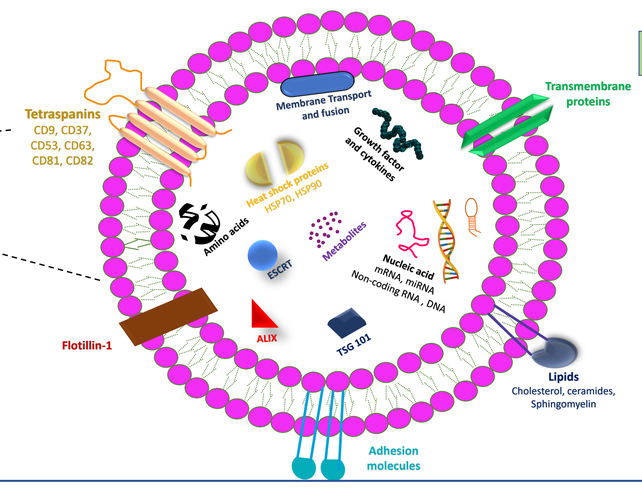




Why liquid Biopsy(serum) and exosomes?

1. Tissue biopsy depends on factors such as patients age, immunity, etc.
2. Tissue biopsy has to be performed repeatedly for monitoring the patients condition. This makes it more complicated in cancers relevant to brain, pancreas etc.
3. Circulating Tumor cells are rare in blood(~10).
4. They enter the blood stream during advanced stages of cancer.

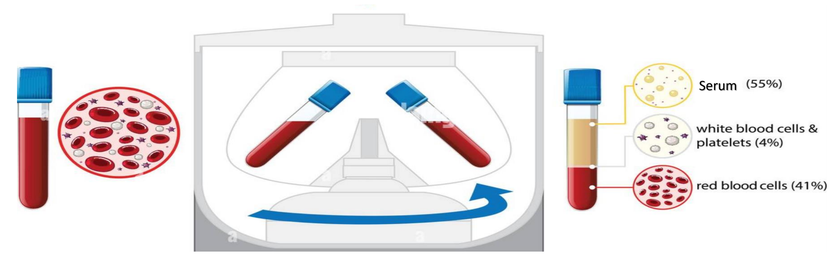




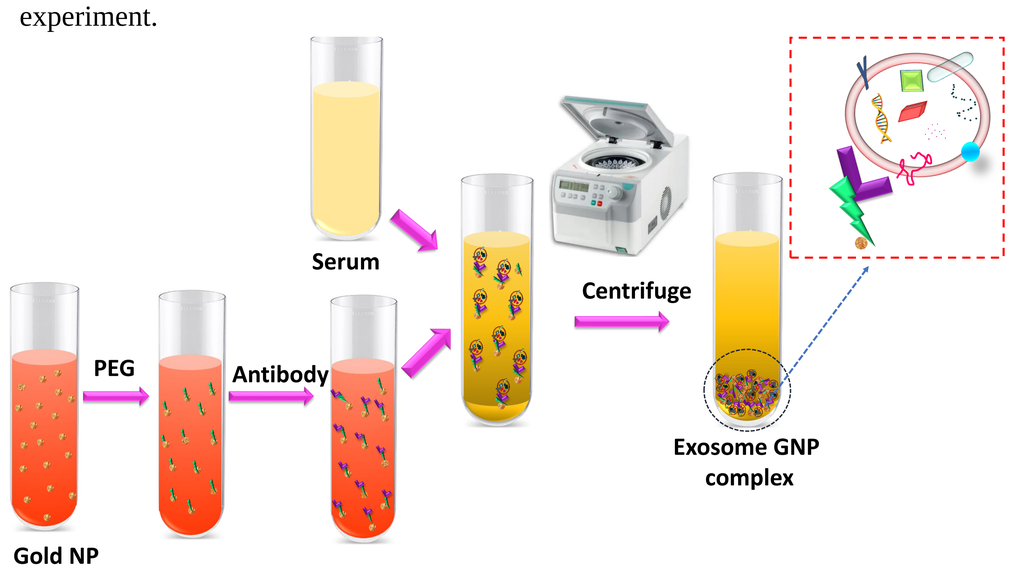
About exosomes

- Size : 20 nm – 200 nm
- Released by cells.
- Abundant in number(~10^{12}/mL).
- Enter blood stream before Circulating Tumor Cells.
- Contents depends on the cell from which they are released.
- Its cargo(proteins,DNA,RNA) can be used for cancer detection.

METHOD

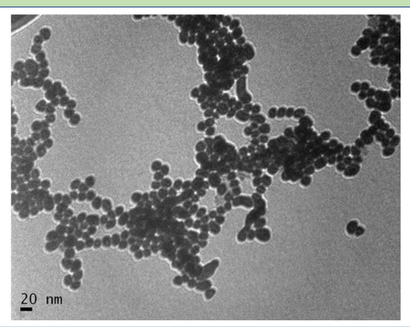


Preparation of Serum from blood. Blood from healthy volunteer is spun in a centrifuge to separate serum from cells. 100 µL of serum was used for each experiment.

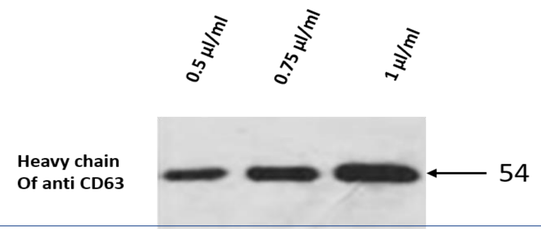


A brief workflow of the conjugation of Gold Nanoparticles(GNP) with exosomes. GNP are prepared by turkevich method. Upon incubation with PEG, it forms PEGylated gold nanoparticle. Anti-CD63 antibody is conjugated to the PGNP to make it suitable for conjugation with Exosomes. When serum and Anti-CD63 functionalized GNP are incubated, Exosome-GNP complex forms, which can be isolated in a tube by centrifuge.

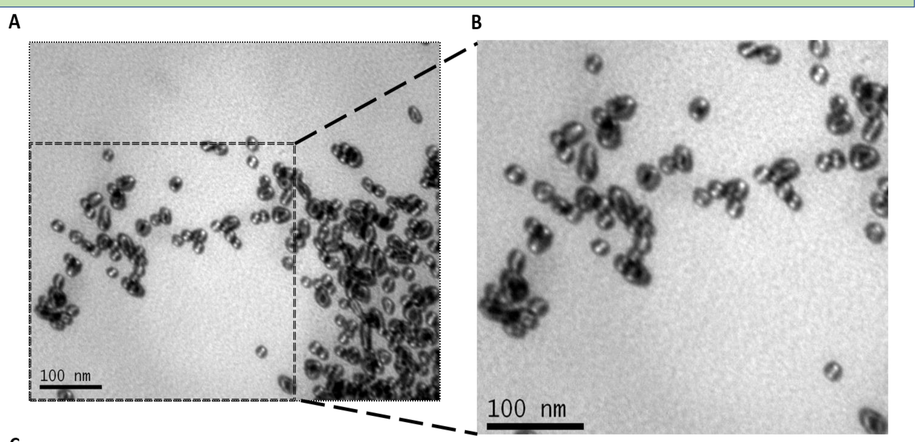
RESULTS

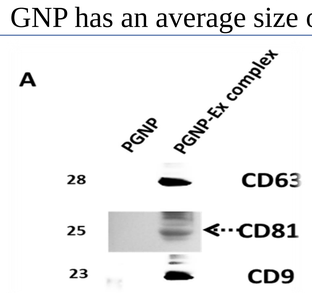


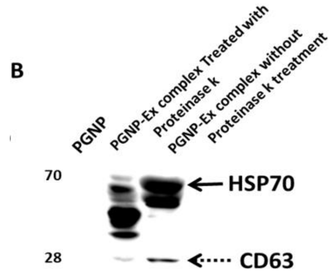
Transmission Electron Microscope(TEM) image of Gold Nano Particles(GNP). Figure shows that GNP has an average size of 20 nm.

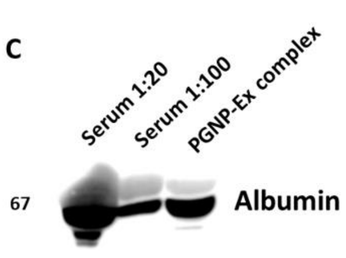


Western blot performed with Gold Nanoparticle(GNP) conjugated with anti-CD63 . The blot shows the western results of SDS-PAGE loaded with GNP conjugated with different concentrations of anti-CD63. The blot is incubated with secondary antibody and developed.

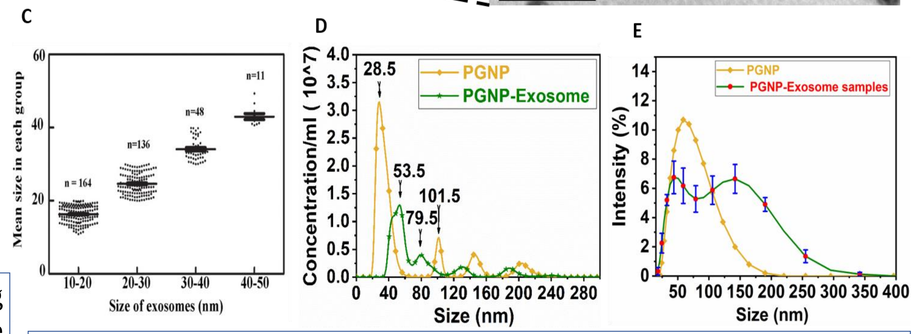








Western blot results of SDS-PAGE loaded with PEGylated gold nanoparticle conjugated with exosomes. (**A**) Blot showing exosomal surface proteins CD9, CD63, CD81 at weights 23, 28 and 25 kDa respectively. (**B**) Blot showing HSP70 and CD63 from the samples with and without proteinase k treatment. (**C**) Blot showing reduced presence of albumin in the exosome sample



Characterization of exosomes with different tools. (A) Transmission Electron Microscope(TEM) image of exosomes. (B) The box in A is shown as zoomed. (C) Grouped scatter plot showing the size distribution of the exosome, with standard error of mean (SEM) ; Size distribution and quantification of PGNP-exosome and exosomes using (D) NTA (E) DLS.

DISCUSSION & CONCLUSION

	Multiple samples easily taken	Duration for results	Requires expertise for analysis	Sample requirement	Advantages	Disadvantages	Purity	Yields
Tissue Biopsy	NO	~25 days	YES	Tissue, which is operated from patient	Traditional method	More painful & harmful	High	High
Ultra centrifugation	YES	16 h	YES	Serum ~60 mL	High sample capacity, gold standard	Need expensive instrument, Damages exosome	Low	Low
SEC	Yes	< 4 h	YES	Serum ~60 mL	Simple, less expensive	Tedious task, low throughput.	High	Medium
Immunomagnetic Beads	Yes	4-20 h	NO	Cell culture medium	Easy to handle, fast isolation	Long incubation time.	High	Medium
Mass Enhancement	**YES**	**2.5 h**	**NO**	**Serum ~ 100 µL**	**Isolates small exosomes, quick and low speed.**	**Requires Bench top centrifuge.**	**High**	**High**

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