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Isolation of Circulating Tumor Cells using Dynabeads

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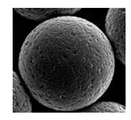
Introduction

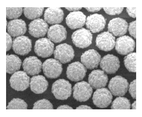
Circulating Tumor Cells

Circulating tumor cells (CTCs) are gaining importance both as prognostic markers and for monitoring of treatment response. The non-invasive biopsies' easy accessibility allows observation of disease progression over time. Due to the low number of CTCs in circulation, highly sensitive methods are necessary for the capture and detection down to single cells.

Dynabeads

Dynabeads™ are superparamagnetic beads that provide an automation-friendly tool for the isolation of circulating biomarkers. Generally, large beads are optimal for working on open platforms while smaller beads are optimal for microfluidics. In this study, Dynabeads with 4.5 µm (M450) and 1.1 µm diameter (MyOne™) were compared for the isolation of CTCs.





Methods

Positive Isolation of CTCs

For positive isolation of CTCs, Dynabeads coupled with monoclonal antibodies (abs) targeting EPCAM (epithelial cell adhesion molecule), e.g. Dynabeads Epithelial Enrich, are used to capture CTCs from whole blood samples.

The captured cells can be obtained in very small volumes and can be lysed directly on-bead for downstream applications such as RT-qPCR or sequencing. The number of beads needed for efficient cell capture depends on the sample volume.

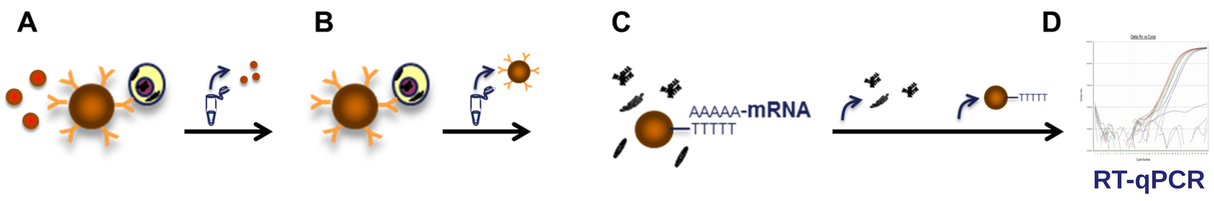


Figure 1: Workflow for positive isolation and detection of CTCs.
A) Epithelial cells are captured from whole blood using Dynabeads coupled to anti-EPCAM abs. The sample tube is placed on a magnet, blood is removed, and cells are washed.
B) Cells are lysed directly on-beads, and the lysate is separated from the beads on a magnet.
C) Lysate is mixed with Dynabeads Oligo $(dT)_{25}$ for mRNA isolation.
D) After washing, mRNA is eluted and analyzed using RT-qPCR. Expression of cytokeratin 19 (CK19), a marker for epithelial cells, is used for cell quantification.

Negative Isolation of CTCs

CTCs can be enriched by depleting CD45-positive leukocytes from the sample using Dynabeads coupled to anti-CD45 abs. Since depletion is independent of CTC surface marker expression, CTCs are untouched and free of beads. Downstream applications include imaging, cell culturing and molecular analysis.

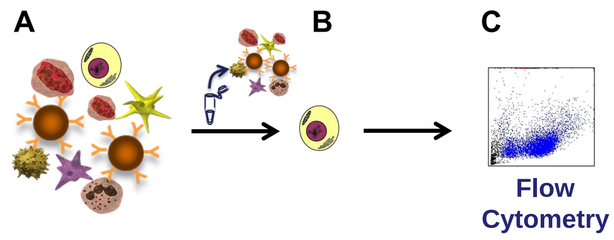


Figure 2: Workflow for negative isolation of CTCs.
A) Leukocytes are captured using Dynabeads CD45.
B) Bead-bound cells are removed from the sample.
C) Remaining cells are analyzed using flow cytometry.

Results: Positive Isolation of CTCs

Sensitivity:

Single epithelial cancer cells were spiked into 7.5 mL whole blood diluted 1:1 in DPBS using a micromanipulator. Dynabeads Epithelial Enrich (M450) were applied for epithelial cell capture (Figure 3). Single cells were also picked and direclty lysed as controls. The Ct values from cells captured using the above workflow were within the range determined by the controls indicating highly sensitive capture down to one single cell.

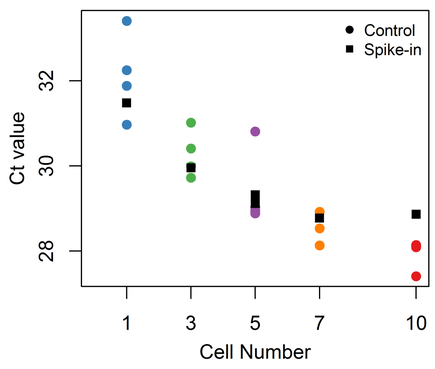


Figure 3: CK19 Ct values obained for different number of cells.
Single cells (1, 3, 5, 7, and 10 cells) were picked and either spiked into and captured from 7.5 mL 1:2-diluted whole blood (black) or lysed directly as controls (colored).

Dynabeads platforms:

Dynabeads MyOne Epoxy coupled to anti-EPCAM abs and MyOne Streptavidin C1 and T1 coated with biotinylated anti-EPCAM abs were compared to Dynabeads Epithelial Enrich (M450) (Figure 4). All Dynabeads platforms were equally efficient in cell capture (A). However, the amount of non-specific binding of leukocytes were lower for the MyOne platforms, especially Dynabeads MyOne Streptavidin C1 and T1 (~10x lower; ΔCt=3.4), compared to Dynabeads Epithelial Enrich (B).

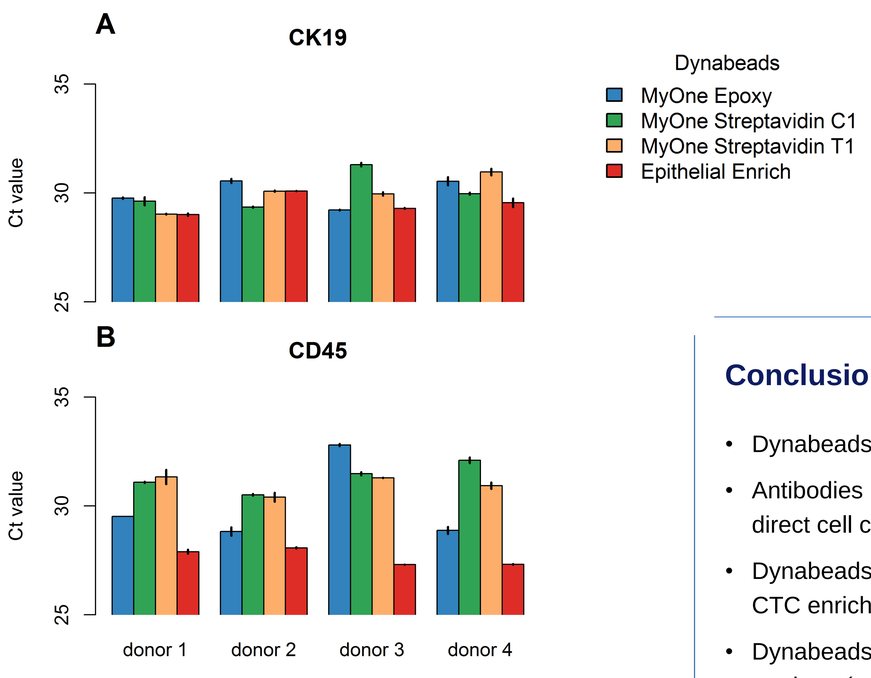


Figure 4: Five cells were spiked into blood of four donors and captured using four types of Dynabeads coupled to anti-EPCAM abs. CK19 (A) and CD45 (B) expression level was analyzed.

Results: Negative Isolation of CTCs

Cell depletion efficiency:

Mononuclear cells (MNCs) were isolated from buffy coat and incubated with Dynabeads CD45 (M450) for leukocyte depletion. After magnetic separation, the cells remaining in the supernatant were stained with PE-labelled anti-CD45 and analysed by flow cytometry (Figure 5).

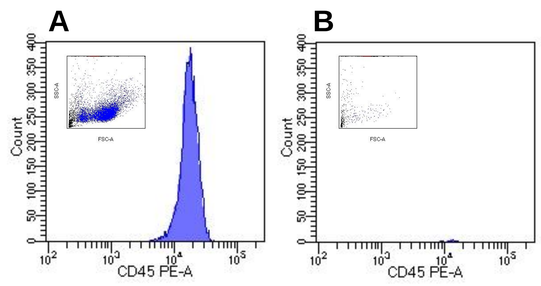


Figure 5: Fluorescence histograms for CD45-positive MNCs and scatter plots analysed using flow cytometry before (A) and after (B) depletion using Dynabeads CD45.

For Dynabeads MyOne, a higher number of beads/cell were required for cell depletion due to the smaller bead size (Figure 6). However, the total bead mass was ~10x lower.

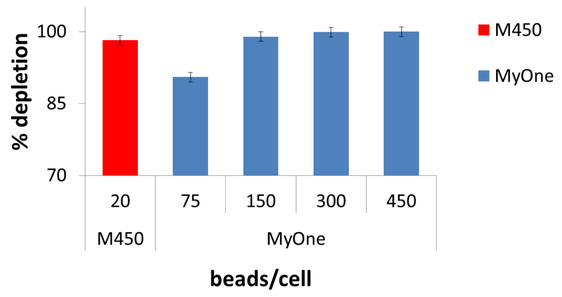


Figure 6: Cell depletion efficiency using Dynabeads CD45 (M450) and Dynabeads MyOne Epoxy coupled to anti-CD45.

Dynabeads platforms:

Different surface-activated Dynabeads MyOne and the corresponding streptavidin-coated beads revealed different depletion efficiencies (Figure 7). Epoxy-activated beads showed the highest depletion efficiency while COOH-activated beads demonstrated least efficient. However, when beads were conjugated with streptavidin, the differences were minor.

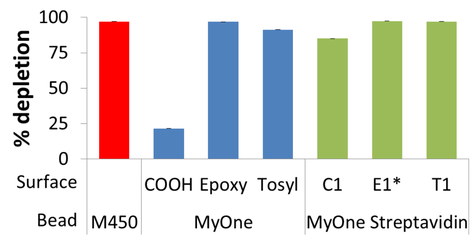


Figure 7: Comparison of cell depletion efficiency using different surface-activated Dynabeads MyOne and MyOne Streptavidin beads (E1*: prototype beads).

White blood cell depletion:

Different sized beads exhibit different binding properties. Small beads have been shown to be beneficial for the depletion of cells with low expression of surface markers.

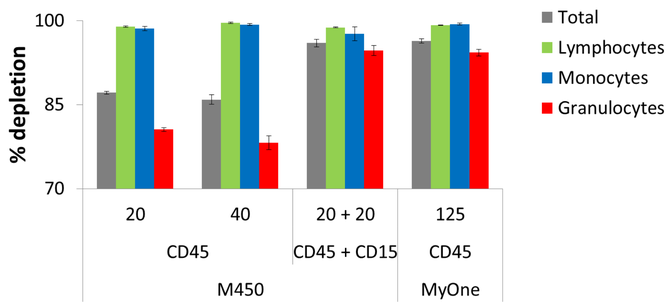


Figure 8: White blood cell depletion efficiency after RBC lysis using M450-beads coupled with anti-CD45, with anti-CD15, and MyOne Epoxy beads coupled with anti-CD45.

Anti-CD45-coupled M450-beads depleted only ~80% of CD45-low granulocytes. Cell depletion improved when M450-beads coupled with anti-CD15 directly targeting granulocytes were added (Figure 8).
In contrast, applying anti-CD45 coupled MyOne beads achieved similar depletion efficiency as the combined use of M450 beads targeting CD45 and CD15.

Conclusions:

- Dynabeads can capture CTCs with high specificity and sensitivity down to single cells.
- Antibodies targeting various markers such as EPCAM can be coupled to Dynabeads for direct cell capture.
- Dynabeads coupled with anti-CD45 efficiently deplete leukocytes from MNC samples for CTC enrichment.
- Dynabeads MyOne are more efficient in capturing cells with low expression of surface markers (e.g. CD45-low expressing granulocytes) than M450 beads.
- Dynabeads MyOne require higher number of beads but less total bead mass to achieve efficient cell capture or depletion in comparison with M450 beads.

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