Application Note

Improved Triple-Negative Breast Cancer (TNBC) model for relevant screening of therapeutics targeting cancer stem cells

- Triple-negative breast cancer (TNBC) has the worst prognosis of all breast cancers.
- The severe prognosis is due to cancer stem cells which result in recurring cancers and few effective treatment opportunities are available.
- TNBC cultured in the ClinoReactor has a more relevant cancer stem cell gene expression profile compared to TNBC cultured in ULA plates.
- TNBC cultured in the ClinoReactor has a more *in vivo* -like cancer stemness ratio compared to TNBC cells cultured in ULA plates, indicating it is a better model to study treatments targeting cancer stem cells.

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Introduction

Breast cancer is the most prevalent cancer affecting women and the second leading cause of cancer deaths among women [1]. Triple-negative breast cancer (TNBC) is the most aggressive form with the lowest survival rate [2]. This application note presents data from an experiment comparing the ability to recapitulate *in vivo* properties of triple-negative breast cancer using the MDA-MB-231 cell line cultured either in ultra-low attachment (ULA) plates or as sodium alginate droplets cultivated in the ClinoStar system.

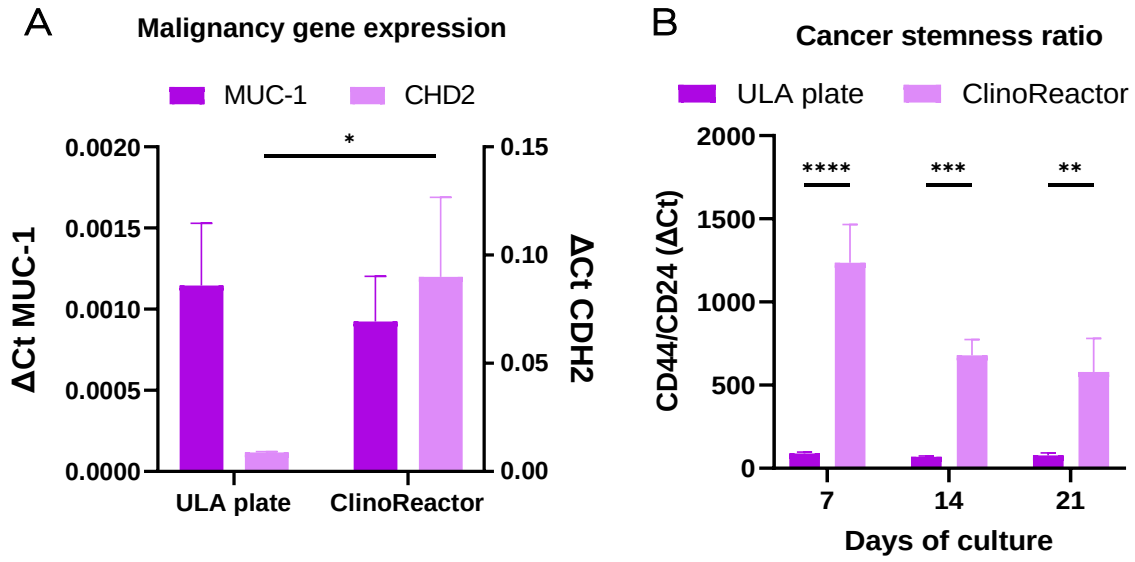

FIGURE 1 Cancer stem cell gene expression and cancer cell stemness A) qPCR data based on Taqman gene expression assay comparing MUC-1 and CDH2 (N-cadherin) normalised to housekeeping gene ACTB. CHD2 expression is significantly increased in the ClinoReactor model B) qPCR based on Taqman gene expression assay comparing the CD44 and CD24 values normalised to ACTB housekeeping gene in relation to each other as an indication of cancer stemness. Throughout the culture period, the ClinoReactor model has a cancer stemness ratio mimicking *in vivo*-like TNBC.

The MUC-1 gene is increased in 90% of breast cancers [3]. Comparing MUC-1 expression between ULA and ClinoReactor cultivation shows similar expression (Figure 1A), hence are both able to recapitulate this specific TNBC characteristic. However, the significantly increased expression of CDH2 (N-cadherin) in the ClinoReactor (Figure 1A) is closer to the levels seen *in vivo* [4]. Gene expression of a more cancer stem cell

morphology is thought to be related to the increased CDH2 expression [4]. Other markers of cancer stem cells are the ratio of CD44/CD24, which is also seen increased in TNBC *in vivo*. Comparing ClinoReactor and ULA plate cultivation clearly indicates that the cancer stemness is increased in cells cultured in the ClinoReactor (Figure 1B), hence is the better model to study treatments that target cancer stem cells.

The changed gene expression towards more *in vivo* like observed in the ClinoReactor model can be due to the higher compactness of the cells which is evident from Figure 2, left. The gel embedding of sodium alginate and dynamic rotation of the ClinoReactor leads to increased cell-to-cell interactions through innate matrix production, which can contribute to the improved *in vivo* mimicry. Additionally, despite the increased compactness of the spheroids, the viability, measured by ATP content per spheroid is higher in constructs cultured in the ClinoReactor after 14 and 21 days (Figure 2, right).

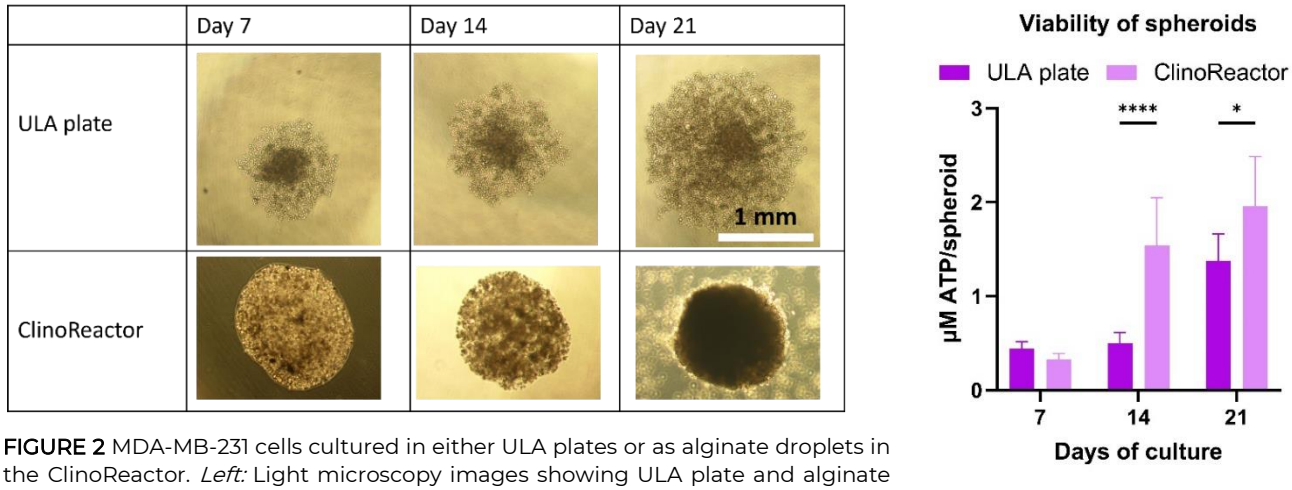

FIGURE 2 MDA-MB-231 cells cultured in either ULA plates or as alginate droplets in the ClinoReactor. *Left:* Light microscopy images showing ULA plate and alginate droplets in ClinoReactor at day 7, 14 and 21 in culture. *Right:* Viability measured as ATP content per spheroid.

Protocol

Cell culture

1. Prepare growth media, sodium alginate solution and cross-linking solution.
2. Sub-culture and count MDA-MB-231 according to ATCC protocol.
3. Seed MDA-MB-231 cells in ULA plates with 5000 cells per spheroid.
4. Create spheroids containing 1 µl 1% sodium alginate with 5000 cells in each droplet and culture in ClinoReactor placed in ClinoStar at 15 RPM.
5. Perform media change every 2-3 days on both cultures.

Gene expression assay

1. Collect samples after 7, 14 and 21 days of culture from ULA plates and ClinoReactors.
2. Extract RNA using RNeasy mini kit.
3. Convert RNA to cDNA using the High-Capacity cDNA Reverse Transcription Kit.
4. Investigate RNA expression using TaqMan gene expression assays according to the manufacturer's manual using MUC-1, CDH2, CD44 and CD24 and ACTB as housekeeping gene.

References

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[2] A. Baranova et al., "Triple-negative breast cancer: current treatment strategies and factors of negative prognosis," Journal of Medicine and Life, vol. 2022, no. 2. Carol Davila University Press, pp. 153–161, Feb. 01, 2022. doi: 10.25122/jml-2021-0108.

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