NK Cells: Marathon Runners and Couch Potatoes

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Introduction

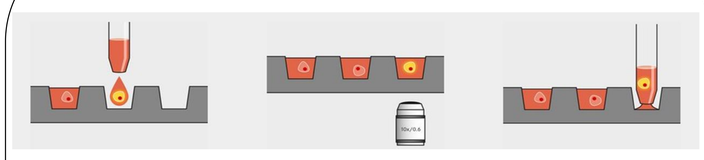
In our bodies Natural Killer (NK) cells play an essential role in the constant fight against cancer and intracellular pathogens. NK cell targeted therapies show great promise to counterbalance immune diseases. However, conclusion drawn from averaged data on the effect of a substance on NK cell activity can often hide outliers and top performers, which could be isolated for further studies.

We wanted to demonstrate the differences in motility in a population of primary human NK cells in a sample. We were able to insert individual NK cells in a controlled micro-scale environment together with RAJI target cells using our novel platform (Fig. 1).

Using fluorescent microscopy and cell labelling we were able to not only observe the NK cell activity, but to also track the NK cells in a time-lapse video format over several hours to quantitatively assess NK cell motility and NK cell killing efficiency inside our microwells.

Over the past decade, tremendous progress has been made in providing tools for high-throughput analysis of large cell populations. However, there is a burning need for technologies that enable the study of large cell populations on the single cell level in an environment that closely resembles *in vivo* conditions, without significantly stressing the cells, while allowing to isolate cells of interest for downstream processes after analysis - all in an economical manner.

Possible applications on nanowells



Dispensing of Single Cells
- high well density
- small volume
- customizable arrays

Live Cell Imaging
- 3 fluorescent channels
- brightfield
- functional assays
- prove monoclonality
- automated cell counting and tracking

Cell Extraction
- Isolate leads alive

Figure 1: Functionalities of the platform.

Methods

The observed natural killer cells were contained in a sample of Peripheral Blood Mononuclear Cells (PBMCs) from a healthy donor, which was kindly provided to us by the medical school Hannover (MHH) and were stained fluorescent blue with Hoechst 33342 (Thermo Scientific). Cells from the Raji cell line were used as target cells and stained with CellTracker Red CMTPX (Invitrogen). We used RPMI + GlutaMAX (Gibco) cell culture Medium with the addition of 200 U/mL IL-2 (PeproTech), Rituximab antibody and fluorescent green death dye SYTOX Green nucleic acid stain (Invitrogen). The PBMCs and Raji cells were combined in a ratio of 1:1. The glas microwell chip was build in House using the patented LIDE technology. Seeding into the microwells and fluorescence time lapse imaging was done on our novel single cell analysis platform, the ARRALYZE CellShepherd. ARRALYZE is a digital cell biology platform that provides three vital features of dispensing, live imaging and extracting single cells by using extremely miniaturized glass-well arrays for functional single cell screening. For this experiment we used wide dimpled 400 µm U-wells (Fig. 2). Such structures do not influence bright field or fluorescent imaging of cells grown in suspension (Fig.3). The environment was humidity controlled and kept at a steady 37°C, 5% CO_2 and the wells were observed over 16 hours.

Cell counting and tracking was done manually. Assessment of viability and NK cell identification was done by fluorescent staining and microscopy.

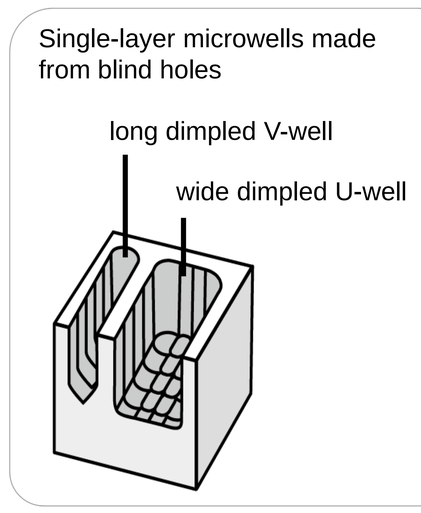


Figure 2: Well structure used in this method.

Results

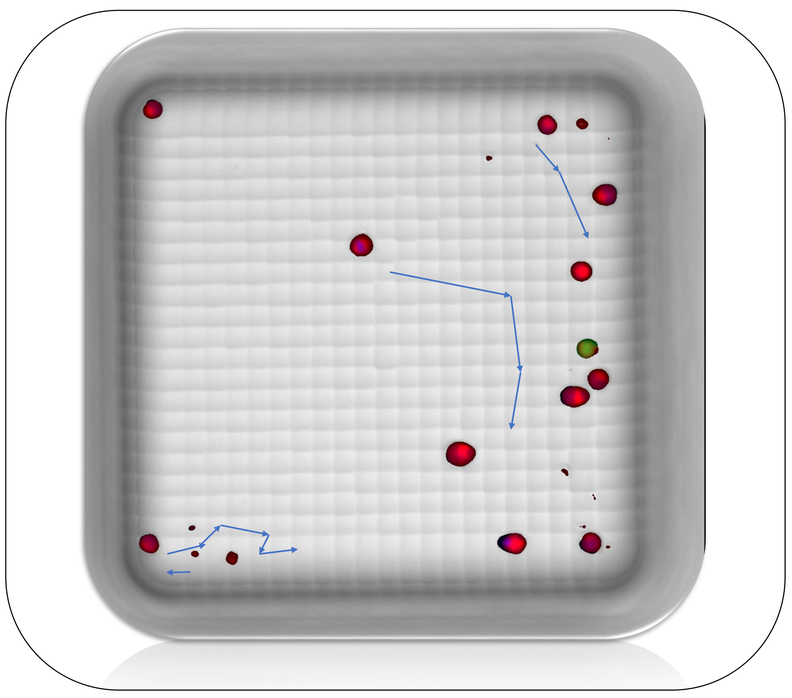


Figure 3: Illustration of NK cell migration in a microwell with a diameter of 400 µm.

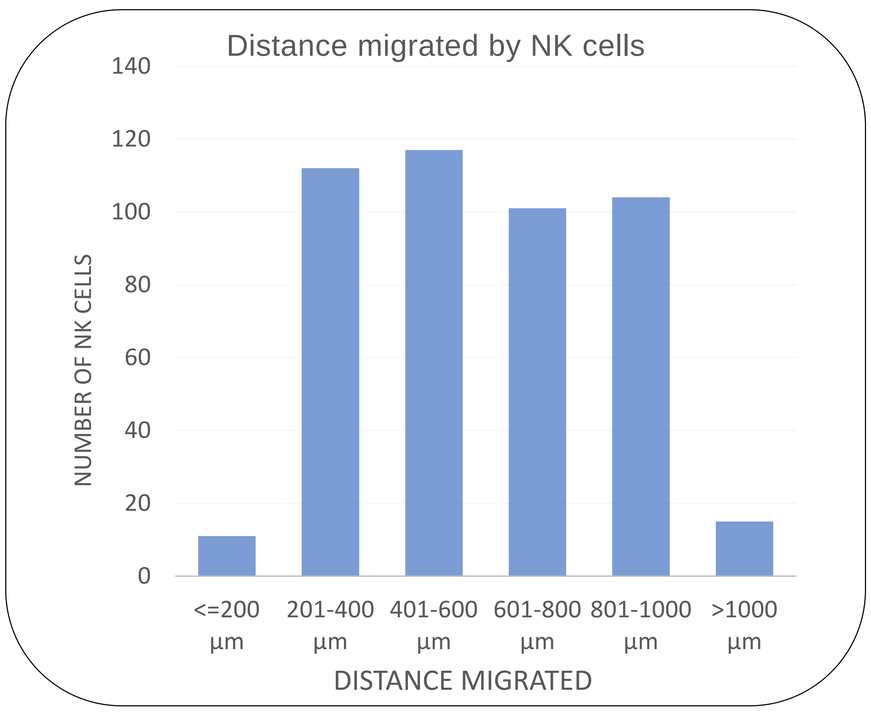


Figure 4: Distance of NK cell migration.

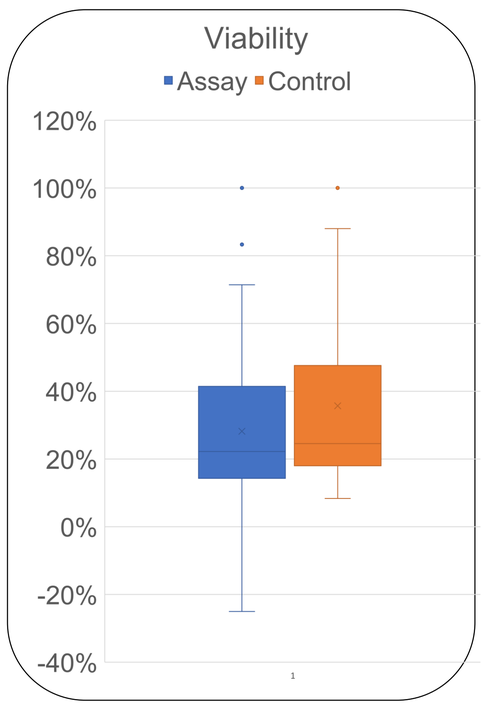


Figure 5: Viability in Assay and Control, counted per well.

In the NK cells migration (illustrated with blue arrows, Fig.3), we observed fast, erratic movement, over short and long distances with swift directional changes. Here the PBMCs were stained with Hoechst 33342 (blue signal); RAJI cells were stained with CellTracker Red; and SytoxGreen was added to the medium to mark dead cells green.

We observed a wide range of NK cell migration distances, ranging from tiny migration confined to one dimple to distances up to 1.7 mm. Considering their size, some of these NK cells really were running a marathon!

The viability of the cells in the control was counted to be slightly higher than in the control, which was also produced inside our platform and incubated in a standalone incubator.

Conclusion

We showed that there is a heterogeneity in the migration behavior within one population of primary human NK cells. To observe such differences novel methods and devices are required. Unmasking as many of these individual characteristics as possible will rapidly aid advancements in cell therapy.

In the future the protocol will be improved by automatic cell counting and tracking, enabled by machine learning methods. Cells of interest could also soon be extracted from the microwells for further analysis or expansion through our cell picking application.

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