MecaChips® soft culture plates by Cell&Soft

APPLICATION NOTE | Efficient cell counting assays using fluidlab R-300 by anvajo

Cell&Soft

SUMMARY

In vivo, cells are usually organized in soft tissues whose rigidities range from a few Pa to a few tens of kPa. However, cell cultures and cell-based assays are still mostly performed on plastic (or glass) surfaces, with rigidities at the scale of GPa, thus more than 10^6 times stiffer than our organs. In order to obtain an *in vitro* response that would allow cells to maintain their *in vivo* characteristics, cell culture devices should more closely mimic the physical properties of the natural microenvironment. Studies have shown that the mechanical properties of the microenvironment deeply impact almost every aspect of cell behavior in many types of cells [1,2]. Therefore, this difference in the physics of the standard culture plates and the soft tissues, can lead to decreased correlation between *in vivo* and *in vitro* results, before any other factor is considered.

Cell&Soft has developed MecaChips®, a new generation of cell culture devices, elaborated with innovative matrices. MecaChips® can imitate the mechanical features of all soft tissues, thus mimicking the microenvironment in which, each cell evolves *in vivo*. MecaChips® offer a new and physiological way to culture cells *in vitro*, far away for plastic culture dishes. MecaChips® culture plates are dedicated to 1. cell culture and cancer research, with the aim of offering cell culture conditions with mechanical features as close as possible to the pathophysiological features of those tissues, 2. stem cell differentiation, to promote the emergence of physiologically relevant phenotypes and 3. drug discovery, to screen drugs in more physiological conditions to raise the relevance of the hits and uncover new molecules more efficiently in clinical practice.

Cell counting is the cornerstone of cell biology. Accurate cell counting and cell viability can be assessed with a variety of methods, that range from manual cell-counting to complex instruments like flow cytometers. fluidlab R-300 from anvajo combines the world's smallest spectrometer with an AI-based automated cell counter based on digital holographic microscopy (DHM). Contrary to brightfield microscopy, DHM does not use optical lenses, which makes the device light and portable. Instead, it generates holograms of the cells which are then automatically processed by convolutional neural networks. fluidlab R-300 uses the label-free DHM technique to analyze cell number and viability directly in the cell culture environment in approximately 15 seconds!

In this proof-of-concept study, we tested the cell counting performance of fluidlab R-300 to monitor the number of cells cultured on soft environment and compared it to the Scepter™ cell counter.

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REFERENCES
[1] Dennis, Discher and al. "Tissue Cells Feel and Respond to the Stiffness of Their Substrate" 310, 1139. Science, 2005.
[2] Ilya, Levental and al. "Soft biological materials and their impact on cell function." Vol. 3, 299–306. Soft Matter, 2007.

METHODS & MATERIAL

Cell culture and preparation

The A549 cell line was plated at a density of 150 000 cells per PD35mm MecaChips® soft culture plates of 4 and 25 kPa (respectively healthy and fibrotic lung rigidities). Cells were allowed to attach and grow at 37°C, 5 % CO_2 for 72 h.

Cell counting assays

Cells were washed with 2 ml of PBS w/o calcium and magnesium for 5 min and detached from the soft substrate by adding 500 µl of trypsin for 5 min. The trypsin reaction was stopped by adding 1500 µl of complete culture medium. The cell suspension was centrifuged for 5 min at 448g and the pellet was recovered in 1 ml of complete medium. Cell number was assessed in pure and diluted (to the half and the tenth) cell suspension in duplicate.

Material Reagents

PD35mm MecaChips® soft culture plates – Fibronectin coating.

fluidlab R-300 automated cell counter.

Scepter™ cell counter.

Dulbecco's Modified Eagle Medium (DMEM) with GlutaMAX 98 supplement with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic.

Trypsin.

RESULTS

Cell counting was performed using the cell counter tool of fluidlab R-300 and the Scepter™ cell counter. The number of cells was monitored at different dilutions. Figure 1 shows exemplary cell images obtained with fluidlab-R300. The cell counter tool of fluidlab R-300 performed better compared to the Scepter™ cell counter because it succeeds in counting cells where the Scepter™ cell counter fails, indicating "too high*" (figure 2). Cell concentration in diluted sample calculated using fluidlab R-300 exhibits a high linearity (figure 3).

Figure 1

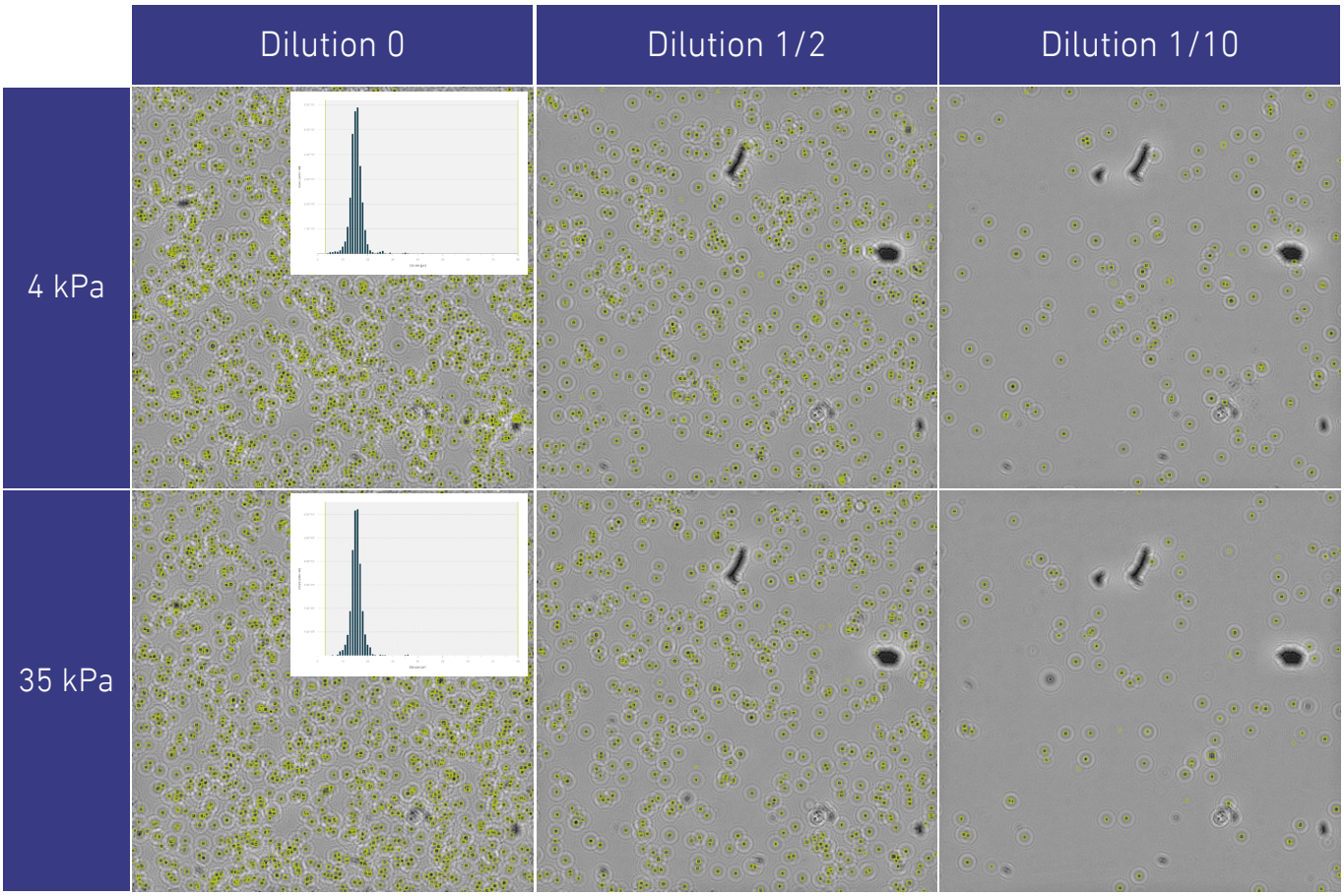

Figure 1: Representative images and histograms of fluidlab R-300 showing A549 cells from 4 kPa-MecaChips and A549 cells from 25 kPa-MecaChips.

RESULTS

Figure 2

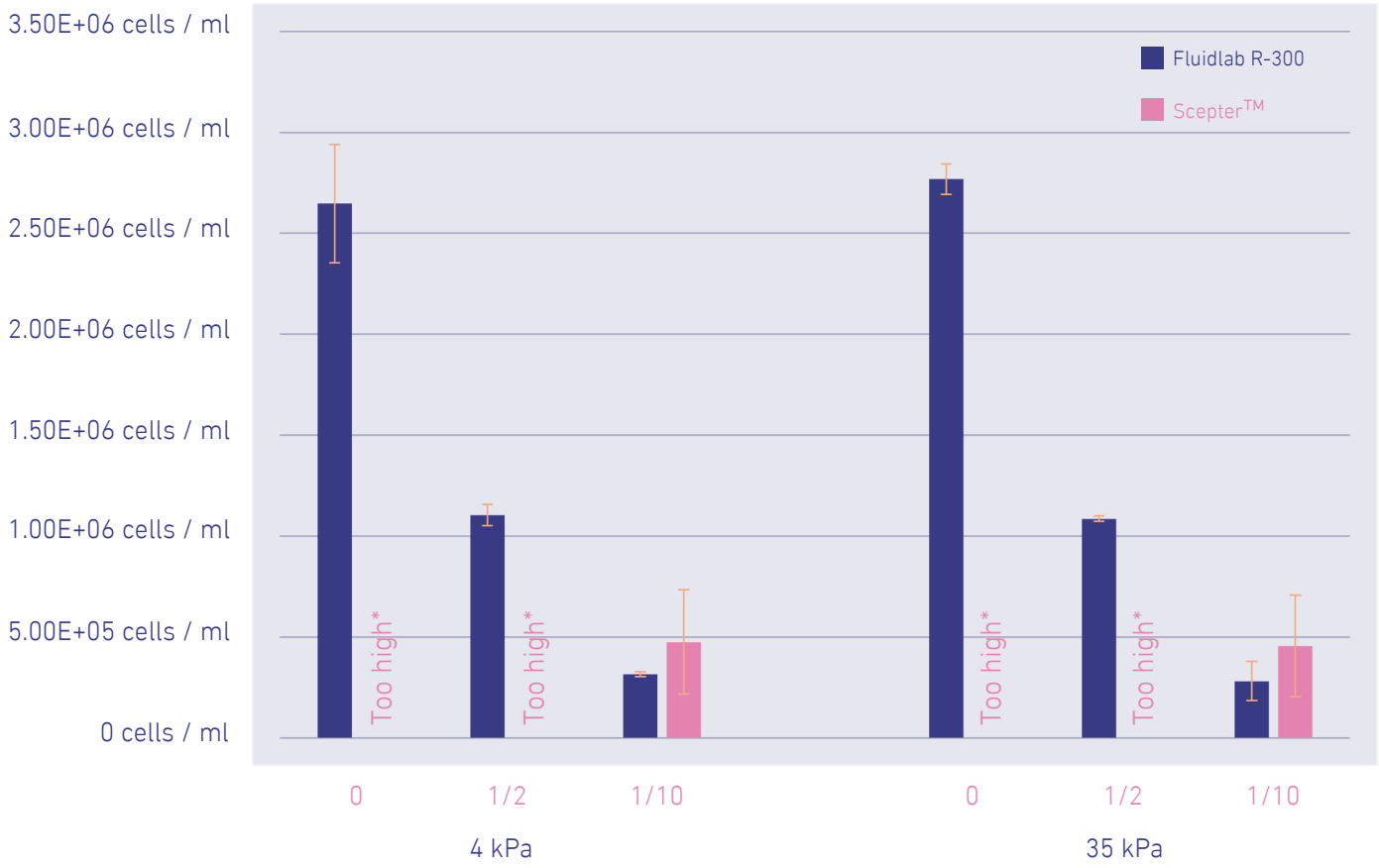

Figure 2: Comparison of different cell counting assays from different sample dilutions. The number of cells were determined using fluidlab R-300 (blue) or the the Scepter™ cell counter (red). Averages and standard errors of the mean were derived from replicates.

Figure 3

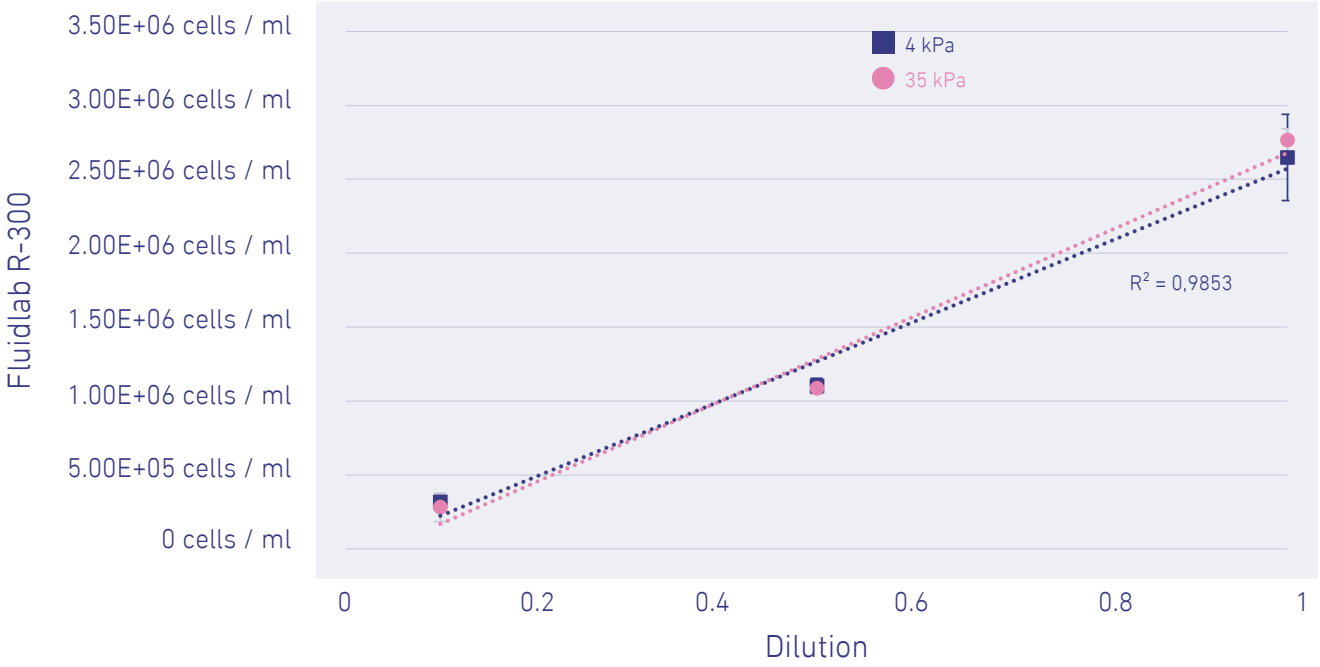

Figure 3: Linearity of serial dilution.

DISCUSSION

Here, we showed that the cell counter tool of fluidlab R-300 can be successfully employed for counting cells grown on a soft environment. The label-free analysis of fluidlab R-300 was developed to work across a large range of cell types and here, we showed that fluidlab R-300 reliably performs cell couting for two different soft substrates with distinct rigidity. We compared its performance to the Scepter™ cell counter. While both devices yield comparable results for the number of cells in the sample diluted to the tenth, fluidlab R-300 offers a distinct advantage: is able to count a large number of cells in a smaller volume (20 µl vs 100 µl for the Scepter™).