Automation of hydrogel-based 3D cell culture

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Introduction

Nowadays drug development relies on high throughput screening involving cell-based assays using cells growing in conventional monolayers (2D). Automation has been adopted in a large number of laboratories in drug discovery to increase the consistency of experiments. However, most of the assays are still based on a system where cells are grown in monolayers rather than 3D formats, even though a high number of citations in peer-reviewed journals have shown an improvement in proliferation, morphology, gene and protein expression levels of cells when moving from 2D to 3D.

To simplify the adoption of 3D techniques in drug discovery, this project investigated the automation of a hydrogel-based modular 3D cell culture system using a liquid handling robot. This approach was proven to be absolutely comparable to manually-produced hydrogels.

Methods

Hydrogel

In this study, the versatile modular hydrogel system developed by Cellendes GmbH was used to encapsulate HCT-116 cells (human colon cancer cell line). In figure 1 the different hydrogel components and the crosslinking reaction are outlined.

The hydrogel-backbone consists of dextran macromers functionalized with maleimides (figure 1A) that are cross-linked under cell-compatible conditions with thiol-bearing linear PEGs (figure 1B). The maleimide-thiol reaction is shown in figure 1C. Cysteine-containing RGD-peptides were also covalently coupled within the hydrogel.

Automation

A Freedom EVO liquid handling robot from Tecan (figure 2) was used to automate the hydrogel preparation. This robot was equipped with an eight-channel Liquid Handling arm with fixed tips. All mixing steps were performed in a reaction vessel (2 ml) or directly in a standard 96-well microtiter plate.

Taxol was used as a reference compound to analyze the drug reactivity of embedded cells in manually- and automatically-prepared hydrogel. Before cell viability and cell growth were assessed by the respective assays, gels were dissolved by the addition of 10 µl of dextranase to each well.

Material

The 3-D Life Hydrogel components Mal-Dextran (M90-3), PEG-Link (L50-3) and RGD-Peptide (P10-3) were used for gel formation.

The cell culture medium used was RPMI-1640: Sigma R8758, supplemented with 10 % FCS, 1 % Pen/Strep. Cell viability was tested using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, G7571) and DNA quantification was performed with the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, P11496).

After cultivating the cells for two, five and eight days, results were analyzed using a multimode plate reader (BMG Labtech, FLUOstar OPTIMA). Pictures were taken using a light microscope (Zeiss Axiovert CFL).

Summary

- **This study showed the easy automation of a novel 3D cell culture system based on dextran.**
- **The preparation was reproducible and reliable using the Freedom EVO platform.**
- **HCT-116 cells showed the typical microsphere formation in the dextran-based gel.**
- **Cell viability was shown to be similar in both manual and automated procedures.**
- **Dose response analysis of Taxol treatment is similar to manual results.**

Acknowledgement

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Results

For comparison, hydrogel was prepared manually following the instructions from Cellendes and distributed into a 96-well plate. The automated preparation of the hydrogel was carried out according to the process shown in figure 3. Both preparations used the same cell density at seeding (10,000 cells/well) in a volume of 50 µl hydrogel. Hydrogels were covered with 200 µl cell culture medium. Light microscope analysis was performed on both preparations after two, five and eight days in culture. Microscope images are shown in figure 4. Two different assays were chosen to compare the processes. The first assay was used to measure cell viability (CellTiter Glo) while the second indicated cell proliferation by the quantification of dsDNA. Results in figure 5 show comparable results from the manual and automated procedures.

A cytotoxic concentration of Taxol (100 nM) was used to determine the accessibility of the hydrogel by drugs and to show the effect of the drug over time, compared to non-treated HCT-116 cells. Results shown in figure 6 demonstrate the cytotoxic effect of Taxol on cells cultured in the dextran-based hydrogel. While non-treated cells proliferate and therefore show an increase of ATP over time, Taxol inhibits cell growth and cell numbers decline under treatment.

Results from the manual and automated processes are similar. A full dose response curve with Taxol was performed in order to verify that the dextran-based hydrogel is suitable for a routine cell-based assay. Taxol was applied in different concentrations, starting on day three after inoculating the cells in the 3 D-Life hydrogel. Cell viability was then carried out at day 8 using CellTiter Glo. Results are shown in figure 7.

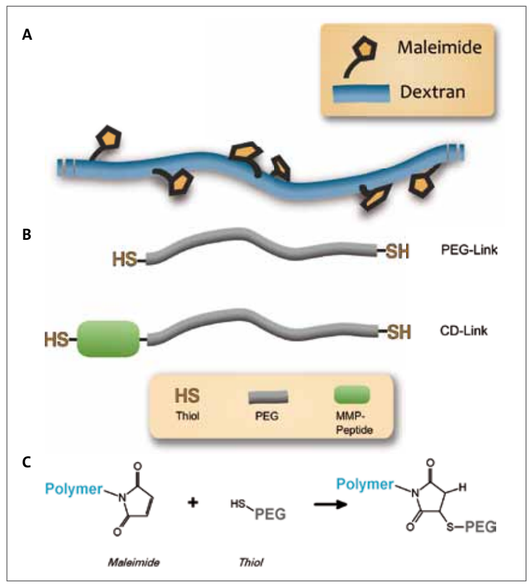

Figure 1: hydrogel components and cross-linking reaction. The maleimide dextran polymer is shown in A), crosslinker di-thiol-PEG with or without matrix metalloproteinase cleavage site in B), and the reaction scheme in C).

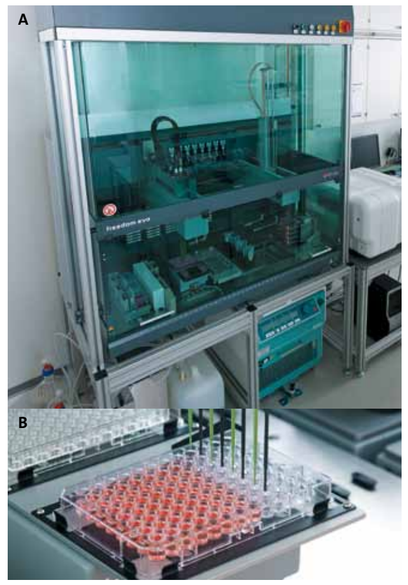

Figure 2: liquid handling robot. In A) the Freedom EVO robot is shown and B) a 96-well plate is filled with medium using fixed tips.

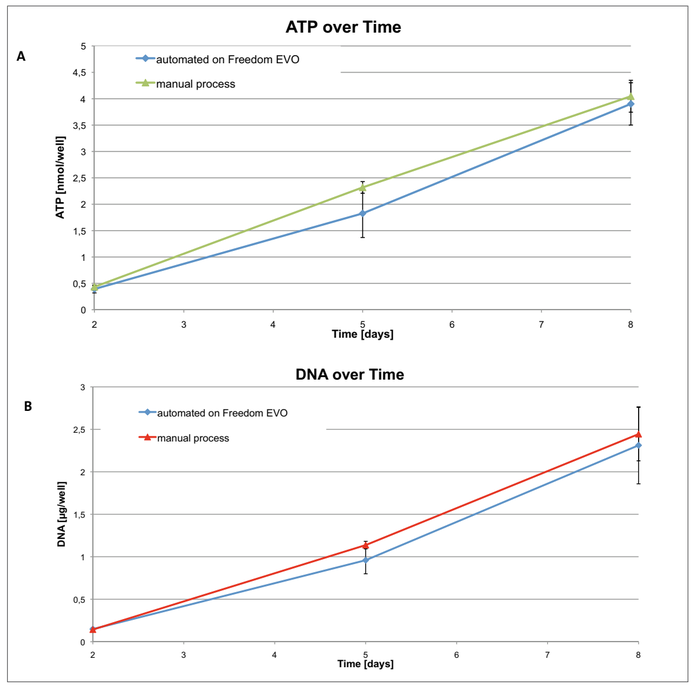

Figure 5: cell culture (HCT-116 cells) in hydrogel: comparison of manual and automated preparation by two different assays at days two, five and eight. A) Viability assay with CellTiter Glo; B) Quant-iT PicoGreen DNA quantification.

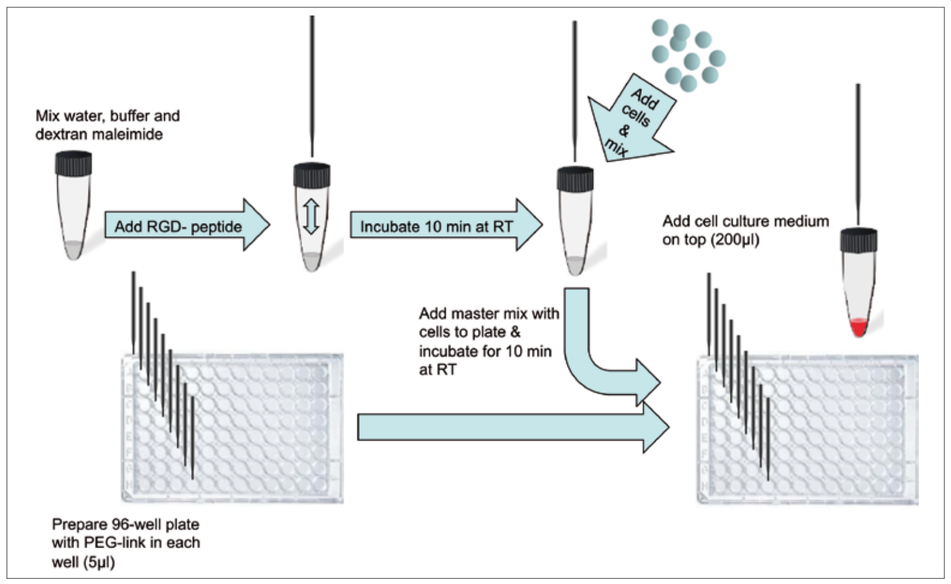

Figure 3: hydrogel preparation on Freedom EVO

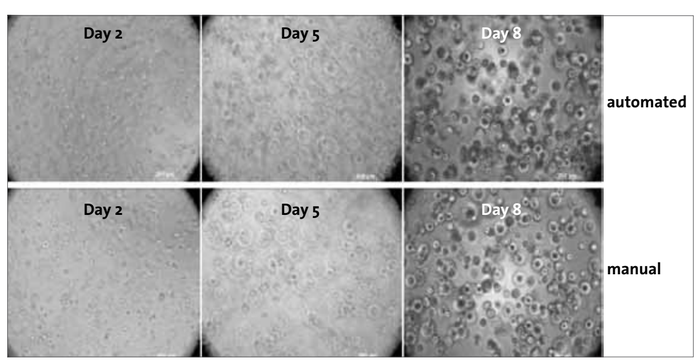

Figure 4: cell culture with HCT-116 cells in hydrogel. The upper row hydrogel preparation and cell plating was automated, while the lower row preparation and plating was done manually for comparison. Seeding density was 10,000 cells/well. Light microscope images were taken at days two, five and eight.

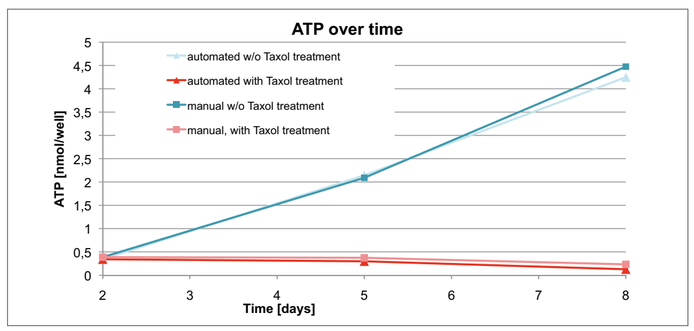

Figure 6: cell culture with HCT-116 cells in hydrogel. Measurements of ATP concentration shown at days two, five and eight, with and without Taxol treatment (100 nM).

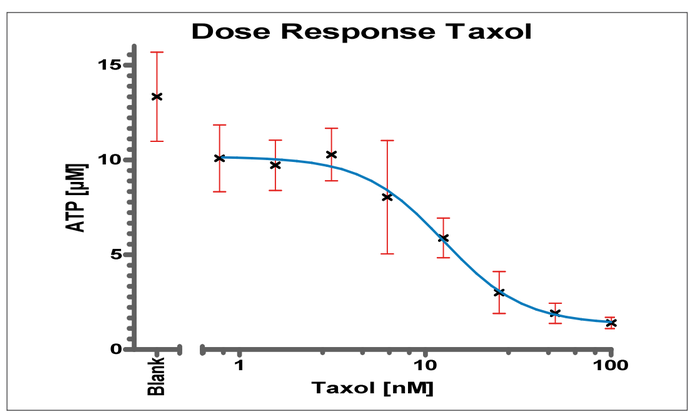

Figure 7: dose response with Taxol on HCT-116 cells in hydrogel. Cell viability check was done using CellTiter Glo, Taxol treatment performed using the following concentrations: 100, 50, 25, 12.5, 6.25, 3.125 , 1.5625, 0.78125 nM.