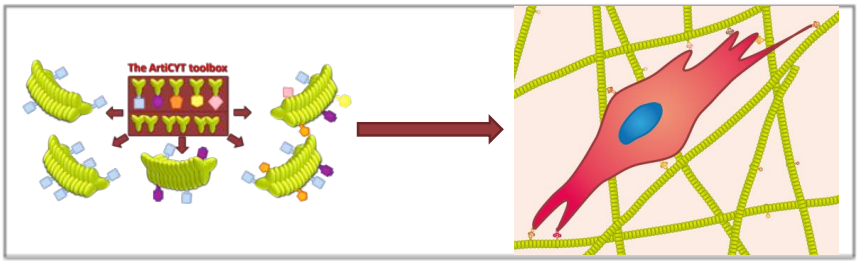
APPLICATION NOTE



ArtiCYT 3D Cell Culture Matrix

Key Features:

- ArtiCYT Bioactive Basal Membrane (BM) 3D Matrix is highly transparent and compatible with multiple staining dyes and protocols employed in High Content Analysis, including stains for live cell analysis.
- ArtiCYT BM 3D Matrix supports High Content Analysis of cytotoxicity in a realistic 3D environment
- ArtiCYT BM 3D Matrix supports formation and analysis of 3D microtissues

ArtiCYT Bioactive Basal Membrane Kit for 3D Cell Culture

Background

Current in vitro models for drug discovery often use cells cultured in 2D mono-layers. It is becoming increasingly clear that these 2D models have serious limitations. More and more researchers are turning to 3D models, as cells cultured in 3D mimic the in vivo situation much more closely. The natural support structure of cells, known as the extracellular matrix (ECM), has a profound effect on the morphological and functional differences between 2D and 3D.

Here, we show that the ArtiCY Bioactive Basal Membrane Kit for 3D Cell Culture , developed using Nano-FM's unique ArtiCYT hydrogel, which consist of synthetic nanofibers that resemble the structure of the natural ECM modified with bio-interactive peptides, support imaging-based live cell cytotoxicity assays and the formation of complex microtissues.

Nano-FM BV

Introduction

Increasingly, complex cellular processes are investigated by multiparametric analysis at the level of individual cells using High Content Analysis (HCA) applications, e.g. to assess cytotoxic events as predictors of human drug toxicity in the drug discovery process.

Here we show that fully synthetic, biomimetic ArtiCYT matrices displaying five peptides derived from laminin, fibronectin, and collagen IV, provide an excellent cell-instructive 3D cell culture environment for encapsulated cells. We demonstrate the compatibility of the ArtiCYT Bioactive Basal Membrane (BM) 3D Matrix Kit with a live-cell cytotoxicity assay based on HepG2 cells. In addition, we show the ability of the ArtiCYT Bioactive Basal Membrane (BM) 3D Matrix Kit to support the formation of more complex 3D microtissues.

Application: cytotoxicity

The liver, as a primary organ for drug metabolism, is the main target of drug toxicity. For this reason, many in vitro cellular cytotoxicity studies focus on liver toxicity. The HepG2 human hepatoma cell line is a common cell-line used in hepatotoxicity models.

We demonstrated the feasibility of live-cell imaging by monitoring cellular response after a toxic insult with acetaminophen (AAP) using different fluorophore dye mixtures. HepG2 cells suspended in DMEM + 10% FCS were encapsulated in ArtiCYT Bioactive Basal Membrane (BM) 3D Matrix at a density of 120k cells in 60 µl gel per well of a 96 well microtiter plate and cultured overnight, followed by incubation with a range of AAP concentrations.

After 24h incubation with AAP, cells were stained with either a cocktail of Calcein AM (20 µM)/propidium iodide (7.2 µM) (Live/Dead stain) for 60 min or for 45 min with a cocktail of MitoTracker Green (1.2 µM), a stain for active mitochondria that enables tracking of multiple early signs of toxicity in high resolution imaging equipment, and HOECHST (1 µM), a cell permeable nuclear dye. Samples were washed with PBS (2 times 5 min) and the cells were imaged on a Leica inverted fluorescence microscope (live/dead) or a Leica confocal microscope (MitoTracker green/Hoechst).

Figure 1 A and B show images of untreated cells and cells treated with 100 mM AAP stained with Live/Dead stain and the graph in figure 1C demonstrates that the number of dead cells increases as a function of AAP concentration. The concentration at which the number of dead cells starts to increase

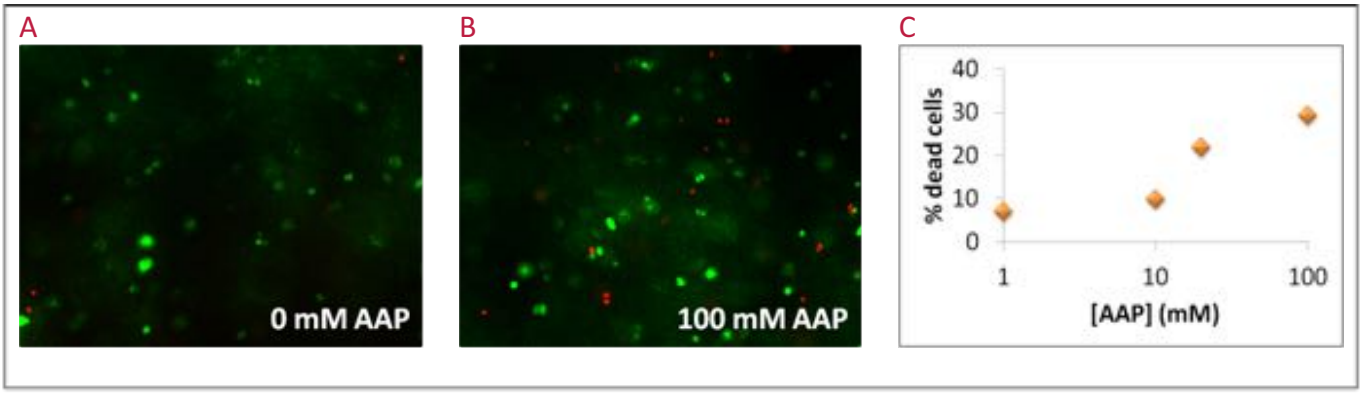


Figure 1: Representative fields of HepG2 cells stained with LIVE/DEAD stain. A: control B: incubated with 100 mM AAP C: graph of % dead cells as a function of AAP concentration.

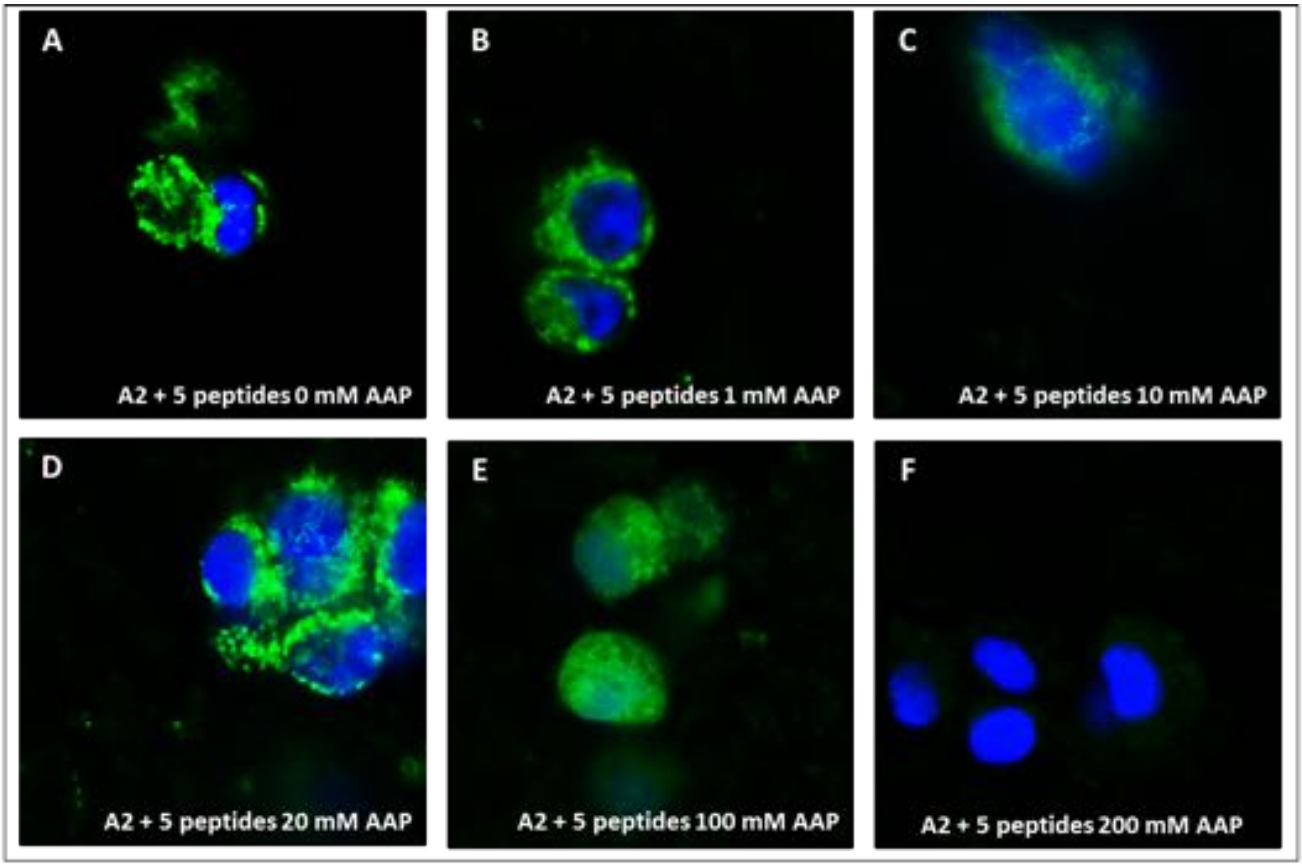


Figure 2: Representative fields of HepG2 cells stained with Hoechst / MitoTracker stain. A: control B-F: incubated with AAP.

corresponds roughly to the known onset of AAP toxicity in 2D HepG2 culture.

Mitochondrial activity provides an earlier indicator of hepatotoxicity. Figure 2 shows cells incubated with 0, 1, 10, 20, 100 and 200 mM AAP and stained with MitoTracker Green and HOECHST. Specific mitochondrial staining of these powerful dyes is clearly visible in the control and concentrations up to 20 mM (Figures 2a-D). The mitochondria can be distinguished as bright green dots in the cytoplasm of the cells. The staining indicates that 100 and 200 mM AAP have profound effects on cell viability as with 100 mM AAP the cytoplasm displays a faint green stain losing most of the granular structure (Figure 2E) and with 200 mM no MitoTracker dye has been taken up by the mitochondria anymore (Figure 2F), clearly demonstrating the usefulness of the ArtiCYT Bioactive Basal Membrane Kit for cytotoxicity measurements.

Application: 3D microtissue formation

In vivo, cells depend on the formation of 3D microenvironments through cell-matrix and cell-cell interactions in order to perform higher level functions. The culture of cells in 3D microtissues is emerging as a more relevant representation of the in vivo situation.

We demonstrated the suitability of 3D microtissue formation using the well established HepG2 model for spheroid formation and MDCK model for epithelial cyst formation. Cells suspended in DMEM + 10% FCS were encapsulated in ArtiCYT Bioactive Basal Membrane (BM) 3D Matrix at a density of 300k cells in 150 µl gel per well of a 8-well Lab-Tek chamber slide and cultured for 14-21 d with regular medium refreshment (HepG2) or 5 d (MDCK). Matrigel was used as control in both cases.

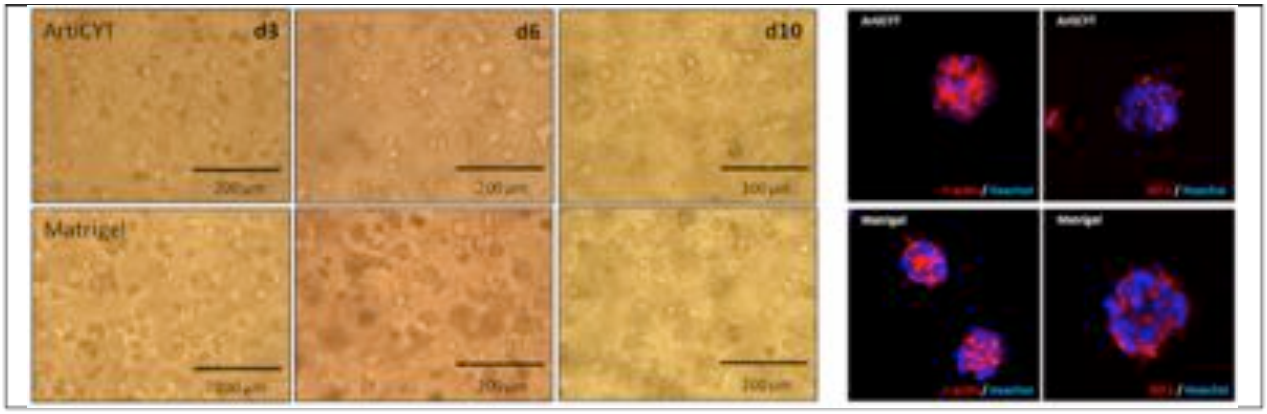


Figure 3: Optical and confocal microscopy of HepG2 spheroid development. Spheroids were imaged with optical microscopy at 3d, 6d, and 10d (left). Confocal images of spheroids stained with f-actin/Hoechst, (middle) and ZO-1/Hoechst (right).

Figure 3 shows optical microscopy images of the development of well organized, dense HepG2 spheroids at several points during two weeks' culture, demonstrating the excellent transparency and bioactivity of the ArtiCYT matrix

At the end of the culturing period the spheroids were fixed using 4% paraformaldehyde (60 min, rt), permeabilized with 0.5% Triton X-100 (45 min, rt), and stained with phalloidin-TRITC (6.6 µM) to visualize their F-actin cytoskeletal arrangement and with specific monoclonal antibodies for ZO-1 to demonstrate the presence of tight junctions. Figure 3 shows that the bio-functionalized ArtiCYT provides the cells with the proper cues to form well organized aggregates with properly developed cell-cell contacts.

MDCK cysts were cultured for 5d and subsequently fixed using 4% paraformaldehyde (60 min, rt). The samples were then permeabilized with 0.5% Triton X-100 (45 min, rt) and subsequently stained using phalloidin-TRITC (6.6 µM) and DAPI (1 µg/ml) (overnight, 4 ˚C), washed with PBS and imaged on a confocal microscope.

Figure 4 shows that even after a relatively short development time, the MDCK cells differentiate and form cysts with densely packed outer surfaces, tight cell-cell contacts, and a well defined lumen in the interior.

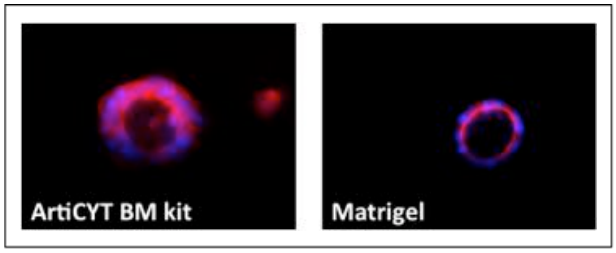


Figure 4: Confocal image of MDCK cysts stained with phalloidin-TRITC and DAPI after 5 d development time.

Conclusion

In conclusion, ArtiCYT has been shown to be compatible with multiple imaging based analysis techniques, including live staining protocols. In addition, ArtiCYT has been demonstrated to be a suitable matrix for the development of acute HCA cytotoxicity assays and a powerful matrix for the development of advanced 3D cell-based models.

Authors

Annemarie Tuin
Menno de Jong

Nano Fiber Matrices B.V.
Groningen, NL