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Application Note

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Sartorius Picus® Nxt Pipette

Case Study on Pipetting Optimization of 3D Biosilk™ Breast Cancer Model

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The interest in 3D models is growing in many research areas, such as cancer research, as they mimic more accurately the cell behavior in real tissue or tumor. Constructing a 3D cell culture model is more challenging than setting up a 2D model, and it often requires testing of several matrices and techniques until achieving the best results. Dr. Caterina Collodet works at the KTH-Royal Institute of Technology (Stockholm, Sweden) and tells us about her work with optimizing a breast cancer model with Biosilk™ 3D matrix (BioLamina, # BS-0101), and how Sartorius Picus® pipette has helped her with her experiments.

You can find more information about Biosilk™ here: http://www.biolamina.com/. In case you are interested in testing the application, please contact Prof. My Hedhammar (Email address myh@kth.se).

Sartorius is happy to partner with scientists developing advanced models of 3D cell cultures.

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Research Summary

The main focus of the laboratory, headed by Prof. My Hedhammar, is to utilize silk assembly to mimic the *in vivo* environment of the extracellular matrix to create functional tissues for biomedical applications. In this context, Dr. Collodet is currently developing an *in vitro* breast cancer model to be used for evaluation of drug candidates and as an innovative system for biomedical research.

What are the main advantages of Biosilk™ compared to other 3D matrices?
The main advantages with Biosilk™ are that the cells are highly proliferative and spread out more efficiently, than when encapsulated in a hydrogel, for instance. Additionally, Biosilk™ can be used to obtain a variety of different scaffolds, such as foams, membranes and fibres. Such scaffolds can also be combined, allowing the creation of co-culture systems.

For the breast cancer modeling, we are still characterizing the model. Preliminary results indicate that cells cultured in 3D Biosilk™ exhibit higher invasiveness markers compared to the same cells cultured in 2D.

Detailed Description of the 3D Biosilk™ Breast Cancer Model in 96-Well Plate Format
The goal is to obtain 3D models where breast cancer cells grow embedded into a Biosilk™ foam. Each foam (thickness ~ 130 µm; diameter ~ 700 µm) is cultured in a well of a 96-well plate as a floating 3D construct (Figure 1). The Biosilk™ foams are kept in culture for a week and used for drug testing, proliferation assays, measurement of gene expression levels and immunofluorescence (Figure 2).

The current major pain point is the low-throughput of the method, which is due to the need to create each foam a) manually using a single-channel mechanical pipette (Sartorius Mline® pipette) and b) onto a Teflon surface from which the Biosilk™ foam is then detached and transferred to a 96-well plate.

We are currently optimizing the foaming protocol to make it high-throughput. In this direction, I am now comparing the 3D Biosilk™ floating format to a "semi-3D format", i.e., where the foam is directly made on an ultra-low attachment 96-well plate and kept attached to the bottom. See the detailed workflow for this increased throughput in Table 1. If we can confirm that changes observed in the 3D Biosilk™ are also present in this semi-3D format, then we will continue using the semi-3D format for future applications. This format would also be compatible with cell culture medium addition using a multichannel electronic pipette, such as the Sartorius Picus® Nxt pipette. As a next step, we are evaluating the 8-channel Tacta® pipette in foaming semi-3D directly into 96-well plate; this seems like a very potential option to increase the throughput and meet our needs for fast mixing speed.

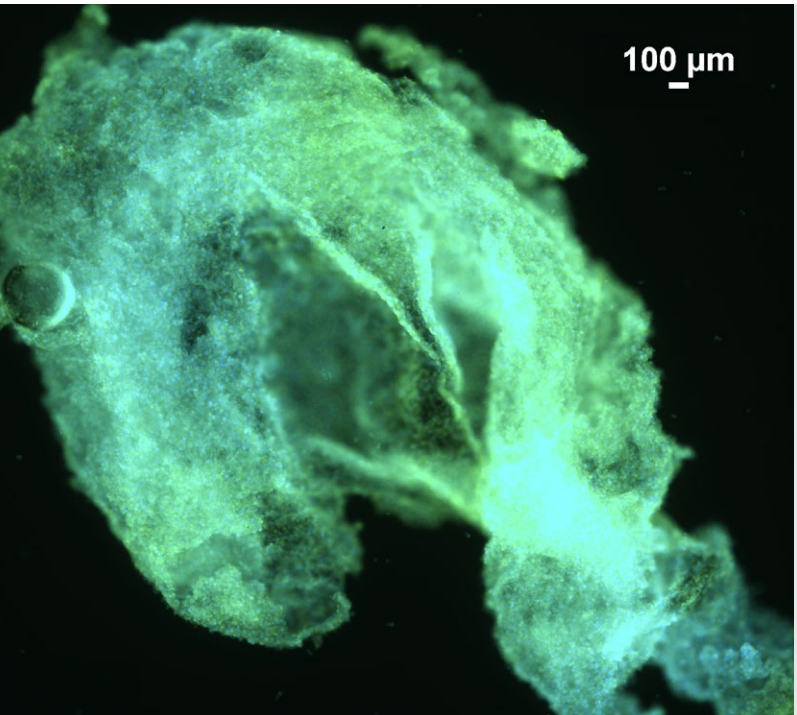

Figure 1: 3D Biosilk™ breast cancer model. MDA-MB-231 cells grown onto 3D Biosilk™ and stained for F-actin with AlexaFluor 488-conjugated phalloidin (green), and nuclei (blue) with DAPI. Scale bar: 100 µm. Image from Dr. Caterina Collodet.

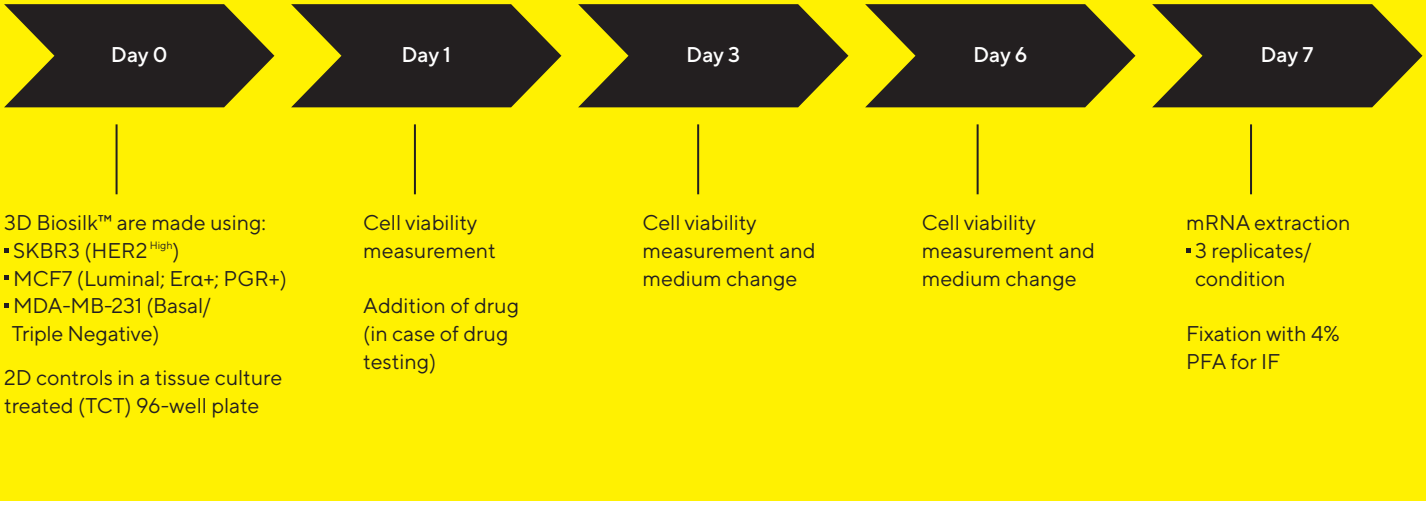

Figure 2: Overview of the workflow for preparation of breast cancer 3D model with Biosilk™. Day 0: Preparing floating 3D cell cultures with Biosilk™ (BioLamina # BS-0101), Day 1: Addition of drug, Day 3: Cell viability test (alamarBlue), Day 7: RNA extraction or fixing cells with 4% PFA for IF microscopy.

What were your main motivations for testing the Sartorius electronic Picus® Nxt pipette?

The main reason was to try a new, more high-throughput protocol for the formation of 3D Biosilk™ foams. The most important changes to the previous protocol were 1, the use of Picus® Nxt pipette (8-channel, 5–120 µL) for simultaneous foaming of 8 Biosilk™ foams and 2, the foaming part directly done on the 96-well plate.

Did you run into any problems or issues with the Sartorius electronic Picus® Nxt pipette?

The pipette speed was likely slightly too low, thus not allowing a foaming comparable to the one obtained with manually controlled pipettes. This resulted in bigger bubbles in the 3D structure, which gave an overall less stable final Biosilk™ foam. An increased speed setting option for the Picus® Nxt pipette could potentially result in tinier bubbles and thus more stable Biosilk™ foam.

What is your opinion of the Sartorius electronic Picus® Nxt pipette?

Sartorius has been our preferred option for pipetting solutions, as we have been working with Mline® pipettes. I was happy to test the Picus® Nxt pipette as it was intuitive to program and, most importantly, it showed clear potential to scale up the production of 3D Biosilk™ foams. Currently the manual foaming of a Biosilk™ foam takes approximately 15 seconds. An 8-channel pipette would allow us to be eight times faster. This would improve the cell survival rate as well as the operator ergonomic conditions.

1.	Resuspend MDA-MB-231 cells in DMEM low glucose 10% FBS 1% PEST at the concentration of 5900 cells/µL. The volumes used for a foam are: 8.3 µL Biosilk™ and 1.7 µL cell suspension (10,000 cells), the master mix of Biosilk™ and cells is prepared estimating a 5% excess.
2.	Thaw the Biosilk™ (BioLamina # BS-0101).
3.	Transfer Biosilk™ to a new 1.5 mL microtube.
4.	Add cells and mix gently 5 times without creating bubbles.
5.	Dispense 10 µL Biosilk™-cell mix onto a 96-well ULA plate (Costar #CLS7007), use a single channel pipette.
6.	Create the foam by mixing 30 times with volume on the pipette set to 18 µL. For this use the protocol on the Sartorius Picus® Nxt (8-channel, 5–120 µL) with volume 18 µL, mixing 30 times, speed 9. During mixing, the pipette tip is kept in the center. Note: In the standard protocol with single-channel mechanical Sartorius Mline® (10-100 µL) pipette, the tip is moved circularly to prevent the formation of large air bubbles (these can create big holes in the center of the culture after bursting).
7.	Incubate plate at 37° C for 20 min.
8.	Dispense with the Sartorius Picus® Nxt 200 µL of cell culture medium to each well and detach the foams from the well bottom with a metal spoon.
9.	Aspirate the medium from the wells carefully (use a manually controlled single channel pipette) without touching the floating foam and apply vacuum with an in-house 3D printed cap (2 x 1.5 min) if possible, in order to burst the air bubbles of the foam.
10.	Dispense 200 µL of cell culture medium to each well and place the plate into cell incubator. After 24 h the foam should appear extracellular matrix-like.

Table 1: Detailed workflow for the semi-3D format with increased throughput (currently being optimized by the Hedhammar research laboratory)

References

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