Optimization of Pancreatic Ductal Adenocarcinoma Organoid Culture

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1. Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer type with a 5-year survival rate of less than 10%. Patient derived organoids are largely coming ahead as potential model to assess therapy efficacy. Current protocols for PDAC organoids generation mostly rely on the use of the commercially available Matrigel. The aim of this study is to explore new methods to improve the organoid formation and uniform their size and shape which is a key aspect when screening the efficacy of different drugs. For this purpose, it was tested if PDAC organoids can be grown in less than 100% Matrigel and if the size and shape can be uniformized by using the SphericalPlate 5D (SP5D). PDAC cells were cultured in the SP5D without Matrigel and in 2%. Organoids of all conditions were collected for histological analyses. The SP5D could be used to culture PDAC organoids but they were not uniformly sized. In 2% Matrigel, organoids did not remain confined in the microwell but rather migrate resulting in the loss of the SP5D's benefit. However, the experiment should be repeated with more PDAC samples to confirm the results. Since the PDAC cases are expected to increase in the future, it is worth to pursue the approaches that were investigated in this study to further optimize the culture of PDAC organoids.

2. Introduction

PDAC is a highly lethal cancer type with a 5-year survival rate of 10%. It develops in the pancreatic duct, which runs through the pancreas [1]. Surgical resection is the only cure but can only be done as long as there are no metastases, which is only the case for 10 – 20% of the patients [2]. Chemotherapy can improve the survival but is not resolutive [3].

Organoids are three-dimensional structures that can be generated in the laboratory from stem cells and cancer cells [4]. Through cell differentiation, they are composed of different organ-specific cell types. For this reason, organoids are representative of the architecture and functions of the parental tissue. They are used in personalized medicine to perform an individual drug screen [5]. A study showed that the response to drugs in patient derived organoids correlates with the in vivo response in those patients [6].

3. Aims and Leading Questions

The first aim of this study is to assess the possibility of using the SP5D to generate uniformly sized organoids. The second aim is to test if organoids can grow in less than 100% Matrigel.

→ Can PDAC organoids grow in less than 100% Matrigel?

→ Can the SP5D be used to culture uniformly sized PDAC organoids?

→ Do the generated organoids resemble to the original tumor tissue?

4. Material and Methods

The SP5D is a plate for three-dimensional culture manufactured by the company Kugelmeiers Ltd. Each well contains a number of micro-wells with a spherical geometry which supports the aggregation phase so that each individual cell is integrated in the organoid formation. This allows the growth of standardized, uniformly sized organoids [8].

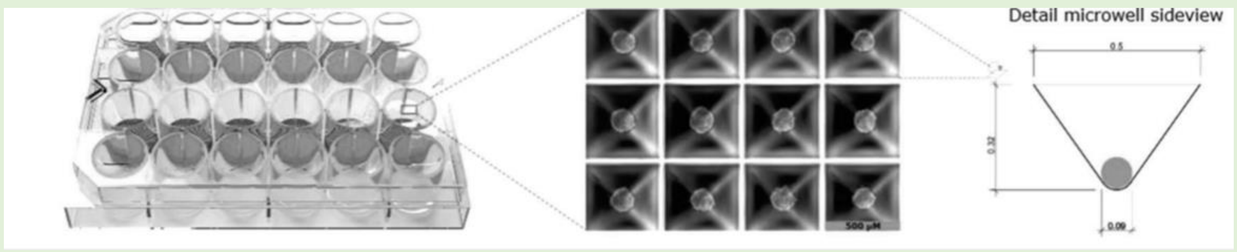

Fig. 1: Scheme of the geometrical structure of the SP5D

Single cells, that were gained from PDAC organoids, were seeded in the SP5D in 2% Matrigel and without. They were incubated at 37°C with 5% CO2. Every day, the culture was checked under the microscope and pictures were taken. Three times per week, fresh medium was added to the wells. After 12 days of incubation, the generated organoids were collected for histological analyses.

5. Results

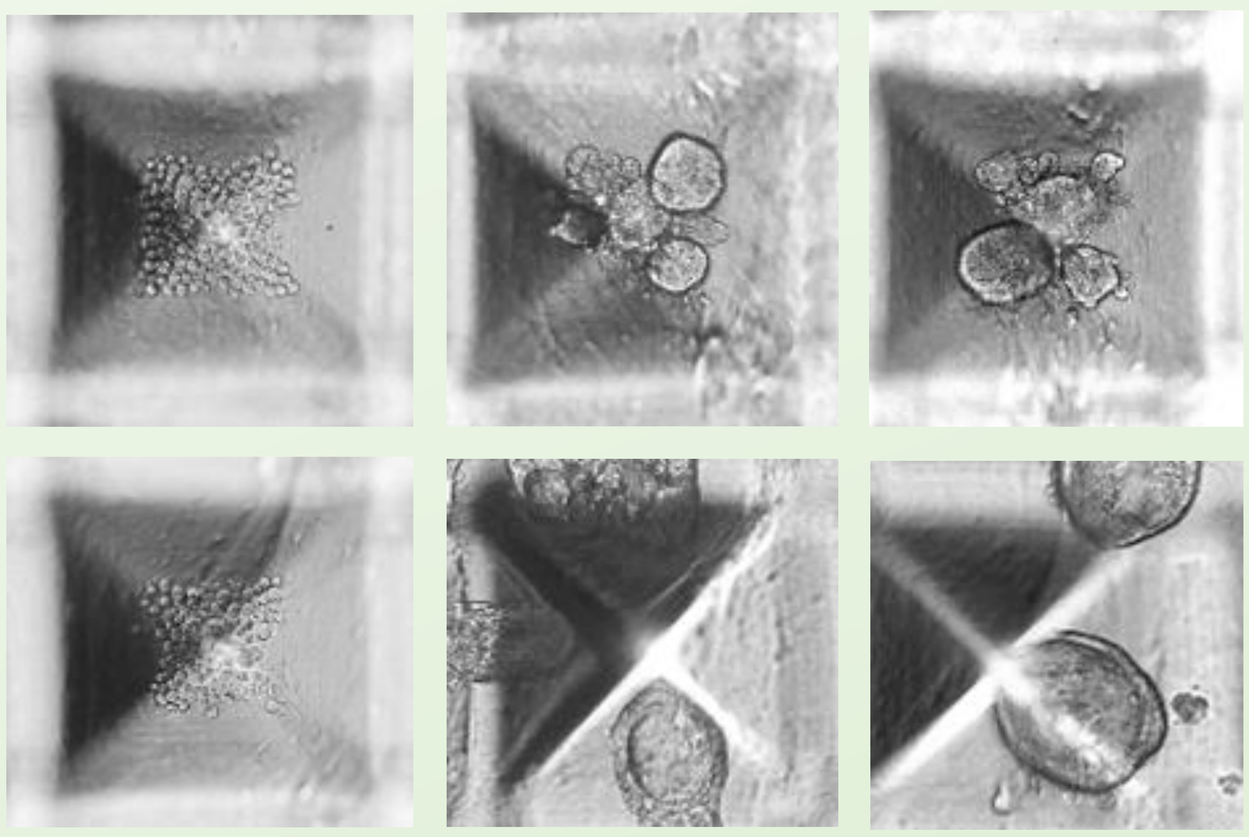

Fig. 2: Brightfield microscopy pictures of PDAC cultured in the SP5D without Matrigel (above) and in 2% (below) on day 0 (left), day 4 (middle) and day 12 (right)

The cells on the seeding day (day 0) were all at the bottom of the well in both conditions. After 4 days, they both formed organoids. While in the 2% Matrigel there are multiple smaller organoids, the ones that were cultured without Matrigel are bigger and started migrating and moving to the surface of the well.

6. Discussion

The experiment showed, that the SP5D could not be used to generate uniformly sized and shaped organoids. Although organoids are forming in both absence and in 2% Matrigel, their size and shape vary. A reason for this might be that there are sometimes multiple organoids per well instead of just one. Another reason might be the migration of the organoids in 2% Matrigel. It seems that because of the movement, the organoids merge together which results in a size variation. To summarize, the SP5D seems to be incompatible with Matrigel for PDAC patient derived organoids. However, since the experiment was only done with one PDAC sample, it is necessary to repeat it with more samples to make a general conclusion.

References

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Figures

Fig. 1 Bornstein et al., 2022, p. 2

Fig. 2 Hänni, M. (2023*). Brightfield microscopy pictures of PDAC cultured in the SP5D without Matrigel and in 2% on day 0, day 4 and day 12* – adapted. medi.