# **Evaluation of BIOMIMESYS® hydroscaffold in mice bearing subcutaneous HCT 116 Human Colorectal tumour cells**

### **Cell culture conditions**

Tumour cells will be grown as monolayer at 37°C in a humidified atmosphere (5% CO2, 95% air). DMEM (ref: BE12-604F, Lonza, Verviers, Belgium) supplemented with 10% fetal bovine serum (ref: 3302, Lonza) and 1% penicillin streptomycin (ref: DE-17-602F, Lonza, Verviers, Belgium). The cells are adherent to plastic flasks. For experimental use, tumour cells will be detached from the culture flask by a 5 minute treatment with trypsin-versene (ref: BE02-007E, Lonza) at 37°C, in Hanks' medium without calcium or magnesium (ref: BE10-543F, Lonza) and neutralized by addition of complete culture medium. The cells will be counted in a hemocytometer and their viability will be assessed by 0.25% trypan blue exclusion assay.

Tumour model development of HCT 116 human colorectal tumours subcutaneously xenografted using Biomimesys®.

## **Cell line amplification**

Briefly, 50 000 cells in 25  $\mu$ l will be incubated carefully seeded on the center of the hydroscaffold in 96-well flat-bottom microtitration plates containing BIOMIMESYS® hydroscaffold. 30 minutes after cells implantation, the wells will be completed up to 200  $\mu$ l as described in the 'first steps' document.

Every 48 hours, 100  $\mu$ l of culture medium will be removed and replace by 100  $\mu$ l of fresh culture medium. Four days after cells implantation, matrix will be transferred in 24-well flat-bottom microtitration plates containing 2 ml of culture medium (to ensure optimal growth conditions).

Every 48 hours, 1ml of culture medium will be removed and replace by 1 mL of fresh culture medium.

#### **Induction of HCT 116 tumours in animals**

According to the number of cells in one hydroscaffold, Then, 8 female SWISS nude mice will be subcutaneously implanted into the right flank with one (D0). The tumour cells implantation with BIOMIMESYS® hydroscaffold will be performed twenty four to seventy two hours after a whole body irradiation of mice (2 Gy 60Co, BioMep, Bretenières, France). Twice weekly monitoring of mice for body weight and tumor growth.

- Daily monitoring of mice for behavior and survival.
- Sacrifice, autopsy (macroscopic examination) after a maximum of 10 weeks after the cell injection.



# **Contact Information**

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