Vascularization and Immune Response within Multi-Cellular Lung Tumor Spheroids

Yan-Shan Xu1,2, Peng-Yuan WANG1,2*
Shenzhen Key Laboratory of Biomimetic Materials and Cellular Immunomodulation, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518055, China1
Institute of Biomedicine and Biotechnology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518055, China2
1068 Xueyuan Avenue, Shenzhen University Town, Shenzhen, P.R.China
Email: py.wang@siat.ac.cn

Background: Tumor is the second highest mortality disease in the world, and tumor metastasis is an important factor affecting the prognosis of patients. Preventing tumor vascularization is one of the methods to prevent and treat tumor metastasis, so it is significant to study the process of tumor vascularization. The function of 3D tumor spheroid is to observe the tumor vascularization which simulate the process of tumor vascularization in vivo, so a vascularized tumor sphere model was designed for convenient of the experiment in vitro. In addition, altering the cell type could set up processes to observe the immune responses in tumor tissue. In the foreseeing future, multicellular tumor spheroids can be used in drug screening for cancer patients, combining with the separation technology of circulating tumor cells, to provide precise treatment for patients.

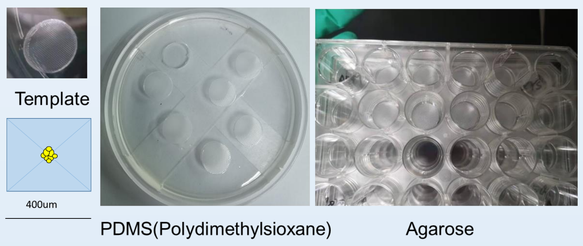
Experiment:

Methods:

Material：
- √ Polydimethylsiloxane(PDMS)
- √ agarose

Medium：
- √ 10%FBS and 1%P/S in DMEM
- √ 5%PL and 1%P/S in DMEM

Cell：
- √ A549



3D scaffold：The custom plastic original mold is structured with neatly arranged inverted pyramid pits, each with a side length of 400um.The reverse mold was prepared by polydimethylsiloxane (PDMS), and then the shape of the original plastic mold was reconstructed by 1% agarose solution.

Cell Culture:

Two different formulations of medium were used for cell culture, 3* 10^4 / mL cell suspension was evenly planted on the three-dimensional scaffold, and no scaffold was used as the control. The oxygen content in the environment where the cells were located was adjusted through the carbon dioxide incubator. The pre-experimental group was in 5% hypoxia environment, and the experimental group was in 2% hypoxia environment, with normoxia environment as the control.

Characterization Analysis

Cell activity and qPCR gene expression were assessed under 8 conditions on day 3 and day 5. Immunofluorescence staining and flow cytometry were performed on the fifth day.

Result:

Fig 1:

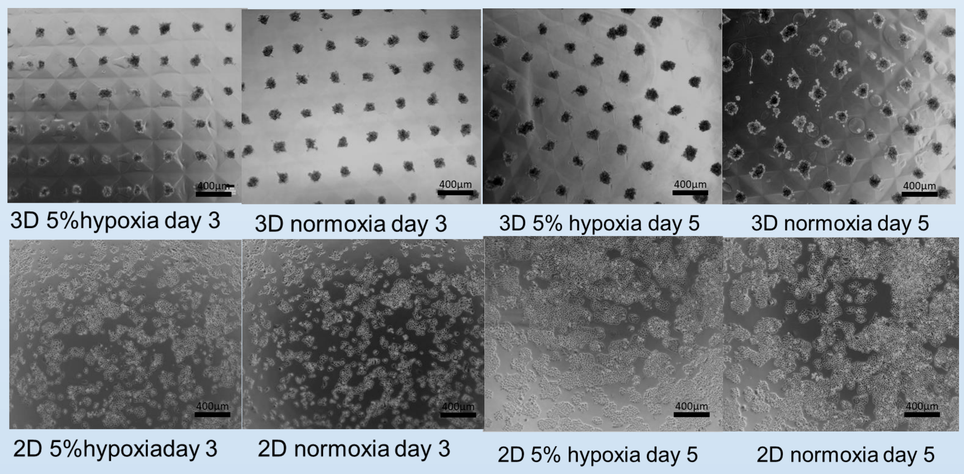
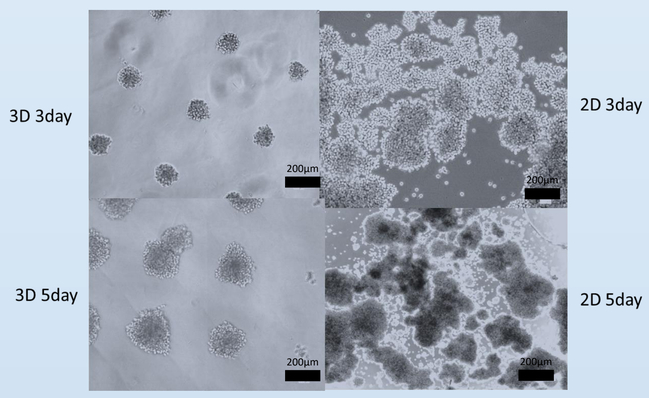


Fig. 1 The cell growth on day 3 and 5 in DMEM medium with 10%FBS and 1%P/S was pretested, confirming that 3D tumor spheroid could still grow under hypoxic conditions. The experimental group adjusted the O_2 condition to 2% hypoxia.

Fig 3:



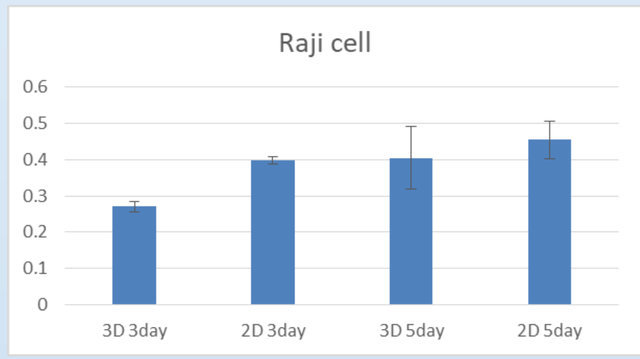
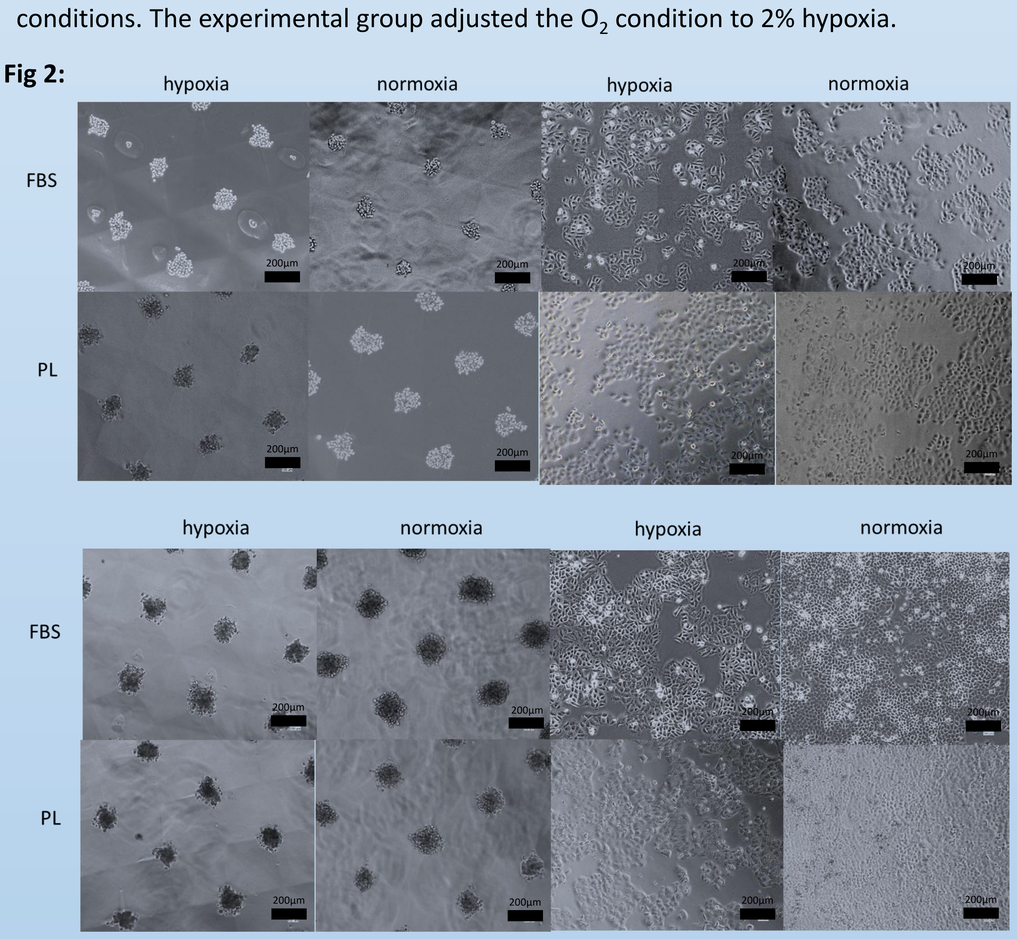
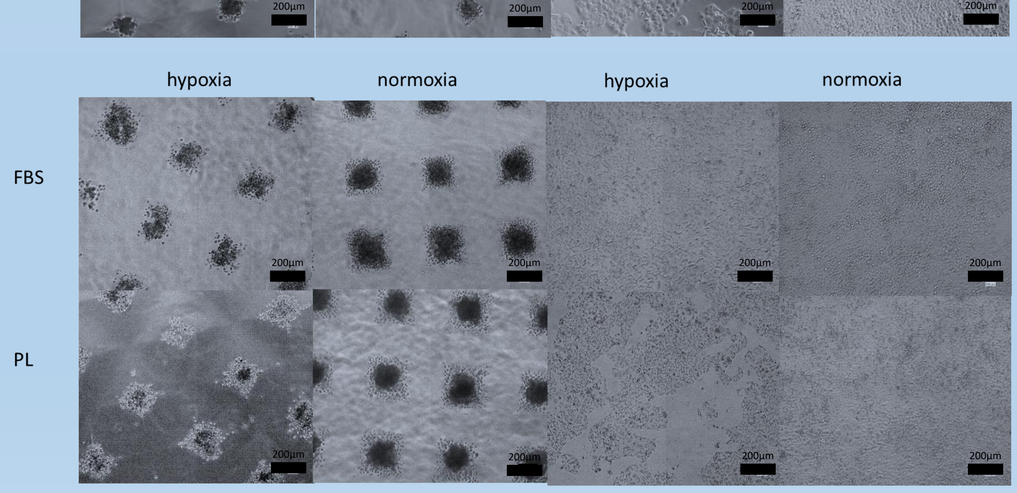


Fig. 3 Similarly, by comparing the Raji cell activity results of lymphoma cells that would naturally form into pellets in 2D and 3D. The cell activity in 3D was lower than 2D, so it could be concluded that the CCK8 cell activity kit would not be inaccurate in measurement due to the inability of reagent to contact the cells due to agarose scaffolds.

Fig 2:





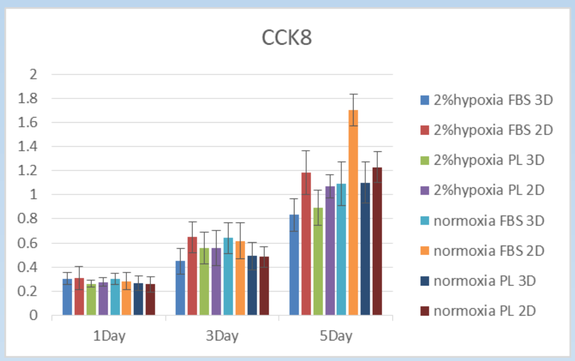
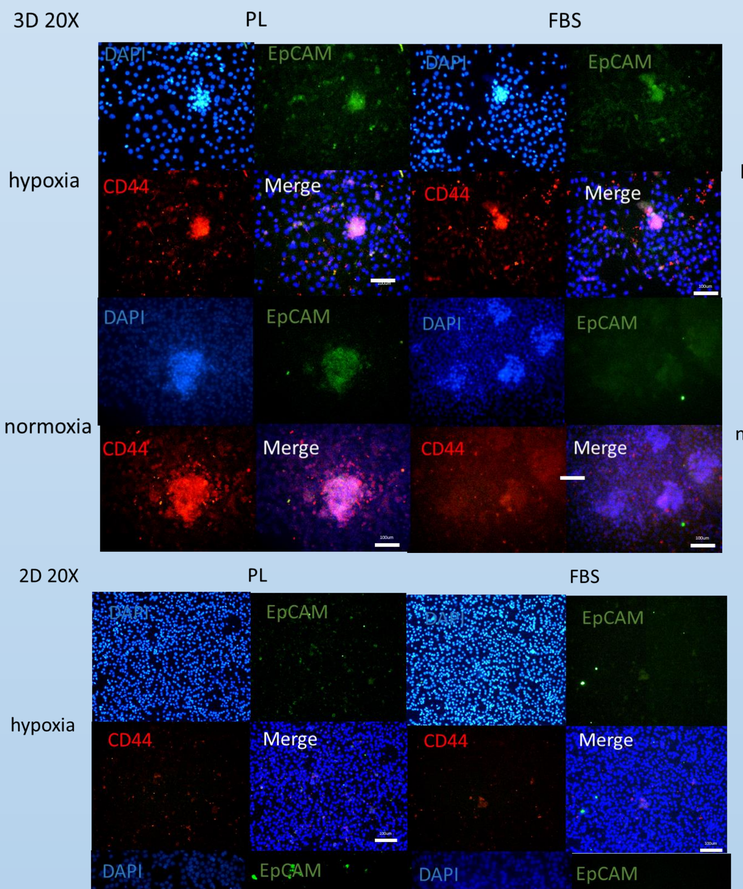
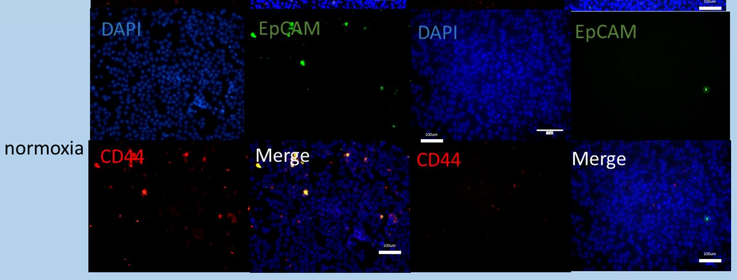


Fig.2 shows the cell growth on days 3, 5, and 8 from top to bottom. Three groups of controls were included: 2D and 3D, 2% hypoxia and normoxia, DMEM medium with 5%PL and 1%P/S and DMEM medium with 10%FBS and 1%P/S. and the last picture is the characterization of cell activity by CCK8 in 3 different days.

Fig 4:





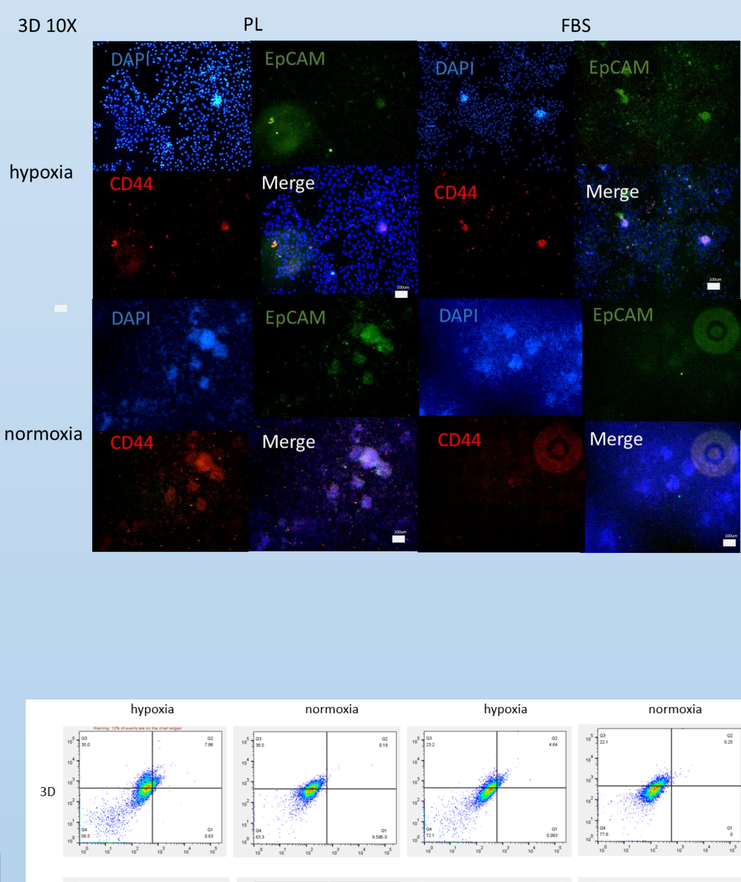


Fig. 5 Tumor Raridity After 5 day of culture, fluorescence staining results showed that the tumor cell spheres in three-dimensional culture expressed CD133 and CD44 proteins.
Excitingly, the 3D tumor cell spheres in DMEM medium with 5%PL and 1%P/S expressed more CD133 and CD44 proteins under hypoxia by flow cytometry. The double positive CD133 and CD44 proteins indicated that the tumor cells under this condition had higher stemness.

Conclusion:

1.The pits of the inverted pyramid on agarose scaffold have a good effect on the formation of 3D cell spheres. Under hypoxia condition, the growth rate of the tumor spheroids in first three days is faster than normoxia, which can be used as a short-term culture condition for tumor spheroids.
2.The 3D tumor cell spheroid in DMEM medium with 5%PL and 1%P/S expressed more CD133 and CD44 proteins under hypoxia by flow cytometry. 7% of cells in this condition show the double positive CD133 and CD44 proteins indicated that the tumor cells under this condition had higher stemness.
3.Cancer stem cells play a very important role in the treatment of cancer, and cancer stem cells with the ability of proliferation in tumor tissues will also have certain drug resistance. If tumor stem cell spheroid can be cultivated for in vitro drug screening through three-dimensional culture method, more accurate treatment can be provided for cancer patients.