Spheroids morphology, cell number, indentation forces and nuclei positioning as responsible for *in vitro* cancer intravasation.

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Abstract

In cancer pathologies, cell intravasation is known as one of the first steps of metastasis. This is described as the release of entrapped cells from the tumors into the blood/lymph fluids, prior to colonize foreign organs.

Due to the dynamic character of malignant pathologies, there is a series of challenges to analyze this step of cancer progression *in vitro* and *in vivo*.[(1)] To this purpose, we developed a new type of hydrogel-based three-dimensional scaffold named "*3D tumor-like microcapsules*". 3D tumor-like microcapsules were defined by their semi-degradable capabilities and their tunable elasticity (mimicking values of stiffness reported *in vivo*). As a secondary relevant characteristic, these matrices have a core-shell morphology. The external shell is mostly constituted by non-degradable and highly crosslinked polymer chains, while the internal bulk is made of a soft tailored-biodegradable hydrogel.

These scaffolds were utilized to entrap the healthy-like cell lines MCF10A and EA.hy926, and the metastatic breast cancer cells MDA-MB-231 and MCF7. Our results show that just cancer cells were able to migrate, proliferate and be released from confinement, while in healthy-like cell lines this behavior was not observed.

Using this system, the generation of cellular aggregates from single cells was studied *in vitro*. Interestingly, spheroids were mostly localized at the scaffold boundary, after a preliminary stage of migration within these matrices. After proliferation, spheroids started exerting forces in radial directions, aimed to disrupt the matrix boundary. After 5 days post-entrapment, indentation forces generated a fracture on the matrix surface, allowing cell release from entrapment (*i.e.* intravasation-like). Remarkably, delivered populations exhibited morphological heterogeneity, with differences in mechanical forces and size.

With the purpose to determine the main biophysical factor responsible for the phenomenon of cell release, we performed experiments in presence of Tissue Inhibitors of Metalloproteinases (TIMPs) and Blebbistatin. Studies done in presence of TIMPs show that cells can still be released, after a short delay in comparison with control experiments. On the other hand, Blebbistatin restricted the generation of indentation forces, and cell intravasation was not observed.

With the purpose to determine the relevance of the nuclei in such events, analysis of cell morphology, nuclei positioning and cell release are currently ongoing.

Preliminary results are showing that aggregates require a minimum number of cells to generate total forces enabling their release. The nuclei positioning, as well as the size of whole spheroids are also two other factors playing a strong role on intravasation-like process, indicating that cell release is indeed a highly coordinated, cooperative and collective process in cancer progression.

References

[(1)] **Leal-Egaña A.**, Balland M., Boccaccini A.R. (2019) Re-Engineering Artificial Neoplastic Milieus: Taking Lessons from Mechano and Topobiology. *Trends in Biotechnology* (in the press).