# How is the ECM affected by autophagy or by different cell culture conditions?

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The extracellular matrix, or ECM is a dynamic network made out of glycoproteins, collagens proteoglycans, as well as other matrisome-associated proteins. The ECM is constantly being remodelled, in response to various stimuli, coming either from the cells themselves or their microenvironment.

One of the cellular pathways possibly affecting the ECM is autophagy. Autophagy is a mechanism allowing cells to survive in stress conditions, such as starvation. Cells break down their own constituents, in order to maintain cell homeostasis and constant protein turnover. Autophagy is mediated by conserved autophagy-related (ATG) proteins, essential for the process, which starts with formation of an isolation membrane, the phagophore. As it closes to form the autophagosome, it sequesters the cargo to be degraded. One protein involved in both steps is ATG7, an E1-like enzyme. This is the reason why in this study, we generated *ATG7* KO cells. By using shotgun proteomics coupled to SILAC labelling for quantification, we were able to compare ECM proteins of WT and autophagy-deficient cells. We found that some proteins could possibly link the highly complex matrix to autophagy.

One external stimulus that may affect the ECM, is cell culture condition. 2D cell culture is commonly used for studying cellular and molecular pathways. However, it does not reproduce *in vivo* conditions closely enough. In fact, animal models are still used in many research domains. An alternative to that would be 3D cell culture, allowing a more realistic environment for cells that were initially taken from a 3D organ. There are already a few known methods that have been tested and approved, i.e. mouse collagen I with matrigel. In this study, we tested another method consisting of magnetizing cells using inert magnetic beads, and then applying a concentrating drive (magnet) underneath to gather cells together into a 3D spheroid. Using a similar quantitative proteomics workflow as the first experiment, we were able to show that cells grown in 2D on a monolayer express more cell-matrix adhesion proteins, possibly due to the fact that they are spread out on a dish and can contact surrounding ECM. Contrariwise, on the 3D spheroid, only cells on the outer layer seem to be able to contact surrounding ECM proteins.

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