2D and 3D cartilage models for drug testing and "high throughput" screening – the impact of PORCN inhibition on cartilage development

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Porcupine (PORCN) is an endoplasmic reticulum protein belonging to membrane-bound O–acyl transferase superfamily. This molecule is necessary for the attachment of long-chain faty acids to WNT proteins essential for their secretion. PORCN is expressed in most tissues of the body and animals with loss of function in PORCN exhibit embryonal lethality at early developmental stages with extensive gastrulation defects. Several dominant mutations in PORCN were described in human patients, who demonstrated significant skeletal abnormalities.

Currently, remarkable progress in stem cell-based tissue modelling has been achieved worldwide, allowing the introduction of human-specific, cost-effective alternatives to animal studies for the pharmaceutical industry. Novel methods of three-dimensional (3D) cell cultures became frequently utilized in many research fields due to their enhanced biological functions as compared to conventional two-dimensional (2D) cultures. 3D cell spheroids or organoids can replicate tissue functions, which enables their use both as *in vitro* models or as a necessary intermediate step in tissue biofabrication approaches. The most promising tool for generating and analysis of 3D cell structures is the recent application of miniaturized platforms or microfluidic chips. Microfluidic technology allows controlled conditions, automatization, reduced amount of reagents and cells, and mainly dynamic conditions for continuous perfusion of nutrients and removal of the metabolic waste of the cells.

In this study, several *in vitro* 2D and 3D methods were established to study the role of PORCN in chondrogenesis in regenerative medicine. Here, we used classical animal models such as primary cell cultures, tissue cultures of long bones, and modern 3D cell culture approaches. Modifying the PORCN function with the LGK-974 inhibitor showed a massive increase in cartilage mass in all model systems. 3D cell cultures were compared to standard culture methods to reflect the benefits of microfluidic platforms. Furthermore, our PDMS platform base can be connected to the syringe pump to reproduce *in vivo* perfusion conditions and increase the value of 3D cell culture.

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