

## **Method for improving functionality of tissue constructs**

We present methods to improve the functionality of engineered tissue constructs using the alignment of cells and extracellular matrix in the living sheet or the optimization of culture medium. We have used aligned sheets to produce three-dimensional tissue constructs that present improved functionality.

A tubular construct, in this case a reconstructed human vascular media (RHVM), prepared with such aligned sheets is first demonstrated. The contractile functions of the RHVM have a greater contractile function than a RHVM made with sheets in which cells were not aligned. A planar construct, in this case a reconstructed human skin equivalent (RHSE) with a dermis, was also produced, using the auto-assembly approach, with aligned skin fibroblast sheets. The mechanical strength and resistance of this planar construct proved to be greater than RHSE made of skin fibroblast sheets in which the cells and the extracellular matrix elements had not been aligned and are distributed randomly indicating that the alignment of skin fibroblasts and extracellular matrix greatly improved the mechanical strength of planar construct such as RHSE.

We also show a method to increase the differentiation level of cells which are present in tissue constructs by using cell proliferation inhibitors, have an increased contractile functionality compared to RHVM cultured without inhibitors.

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