# INKJET PRINTING TECHNOLOGY AND BIO-INK DEVELOPMENT FOR THE BIOFABRICATION OF IN VITRO 3D TISSUES

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Key Words: Inkjet, Bio-ink, Hydrogel, Biofabrication, 3D cell printing

Bioprinting is an attractive technology for the construction of three-dimensional (3D) tissues to be used in regenerative medicine or *in vitro* screening applications. Particularly, piezoelectric drop-on-demand inkjet heads are expected to allow the arrangement of living cells with high precision. However, implementing a biofabrication process based on inkjet printing requires the consideration of several specific issues: first, nozzle clogging and loss of cell viability due to the sedimentation of cells inside the printing head; second, the need for selecting suitable bio-ink materials that would be compatible with droplet ejection at low viscosity and rapid tissue assembly.

To address the first issue, we have developed a novel inkjet head specially designed for ejecting live cell suspensions. Droplets are formed by membrane vibrations generated by a pulse-driven piezoelectric actuator. Soft vibrations during the non-ejecting period also prevent cell sedimentation inside the chamber. Good stability of ejection has been thus achieved with over 90% viability when using human fibroblasts or endothelial cells.

Next we have demonstrated the feasibility of constructing stratified mille-feuille like structures, composed of 2-10 cell layers with a total thickness of 50-300 um, by alternating cell suspension and hydrogel deposition. We are also investigating new bio-ink materials to improve tissue functionality without compromising reliability of ejection. For example, incorporation of submicron-sized gelatin beads into alginate gels has been shown to improve cell adhesion to printed substrates.

These results show that inkjet printing can be effective for designing fine 3D structures and would be a promising tool either alone or in combination with other biofabrication strategies.