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## **Bioprinting: Development of a novel approach for engineering three-dimensional tissue structures**

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Bioprinting is a tissue engineering technique in which spherical cell aggregates, the "bio-ink", are deposited into biocompatible hydrogels, the "bio-paper", by a 3-axis "bio-printer". The aggregates can be deposited into essentially any 3D configuration, and when comprised of adhesive and motile cells aggregate fusion occurs. This self-organizing, liquid-like nature of these tissues is described on a molecular basis by The Differential Adhesion Hypothesis (DAH). The techniques we have developed are quite unique because of the high degree of automation that has been incorporated into our processes and the variety of engineered tissues that we are capable of creating. Despite automation, the creation of aggregates remains a nontrivial and time intensive process. The entire process of aggregate formation from initial cell culture to mature aggregate ready to be loaded into the printer takes approximately five days. This time is a limiting factor in the potential use of bio-printing as a source of on-demand tissues for clinical applications. A solution to this potential problem lies in the cryopreservation of aggregates. Freezing mediums and freezing protocols were tested and the effect of the freezing process on aggregate fusion was determined. An alternate solution to expedite the bioprinting process could lie in the printing of cell 'sausages', tightly packed cylinders of cells. In this method aggregate preparation is forgone. Elimination of this step could allow for increased time in tissue creation. Cell sausage printing provides another technique that could be incorporated into the fabrication of complex tissues. Our experiments in this novel and developing technology of bioprinting represent steps towards building complex tissues via self-assembly.