23] Laser- and chemical-induced fusion to reverse-engineer the physical size of hESC-derived CMs: a non-genetic approach for driven physiological hypertrophy and maturation.

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Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) can self-renew while maintaining their pluripotency to differentiate into cadiomyocytes (CMs), providing a potential unlimited source of donor cells for replacement therapies. However, substantial hurdles remain. For instance, hESC-CMs have small physical size (~10x<adult CMs) and immature functional properties. Adult CMs are bi- or multi-nucleated, ~200μM in length and ~2-300pF in size; by contrast, hESC-CMs are typically ~10-15 times smaller, often mono-nucleated, and do not grow in physical size by undergoing physiological hypertrophy even after long-term culturing (>150 days). Indeed, bi-nucleation of CMs has been suggested as an evolutionary adaptive response in metabolically active cells to double RNA for protein synthesis. Developmentally, bi-nucleated CMs arise from the absence of cytokinesis after karyokinesis during the final round of (incomplete) cell division, followed by physiological hypertrophy. This contrasts the fusion of skeletal muscle myoblasts to form multi-nucleated myotubes. Here, we pursued a combination of laser- and chemical-based induced fusion to reverse-engineer physiological hypertrophy of hESC-CMs for increasing their physical size. By employing optical tweezers that exploit a focused laser microbeam platform, we fused hESC-CMs to construct larger bi-nucleated hESC-CMs in a multi-step process: First, two optical traps were utilized to position two hESC-CMs such that their point of contact is located at the laser scissors' cutting spot. Upon irradiation, an open fusion pore forms then enlarges, with the membranes merging and the fused product gradually rounding up to become a "heterokaryon". Similar outcomes could be accomplished by polyethylene glycol-induced fusion of LV-MLC2v-GFP- and mCherry-labeled hESC-CMs to generate hypertrophied yellow heterokaryons. Electrophysiological experiments further showed signs of Ca-handling maturation. Not only do our results shed developmental insights but generation of adult-like cells without genetic manipulations is of pragmatic significance for translating into cell-based therapies and other applications (e.g., accurate disease modeling, cardiotoxicity and drug screening).