whereas Yamada et al. (2014) could not replicate the finding—one hSCNT-ESC line was derived from an oocyte that was picked from among 31 oocytes donated at once by a single donor. Overall, these data indicate that the oocyte cytosols of different women differ in important ways that go beyond the donors' ages. Finding the specific reason why some women have better cytosol than others could have a huge impact in the reprograming field and, more importantly, in the ever-growing field of human-assisted reproductive technologies. To find the answers, though, we will have to rely on SCNT, a technique in which at least one variable, the genomic DNA, is fixed; only then can we really test the reprograming potential of oocyte-cytosols from different women.

Human SCNT, a technique that not so long ago was considered passé, is now back in the toolbox; this time, at least for the task of finding the best human egg, it will not be replaced by induced pluripotent stem cells.

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Stem Cells Go Soft: Pliant Substrate Surfaces Enhance Motor Neuron Differentiation

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Derivation of motor neurons from human pluripotent stem cells is inefficient and requires complex culture protocols. Recently in *Nature Materials*, Sun et al. (2014) report that differentiating human pluripotent stem cells on soft substrates increases the efficiency of mature motor neuron differentiation by altering cytoskeletal mechanotransduction through the Hippo/YAP/Smad pathway.

Efficient derivation of motor neurons (MNs) from human pluripotent stem cells (hPSCs) is limited by our current understanding of the mechanisms underlying MN differentiation as well as drawbacks arising from inefficient and lengthy existing protocols. Human MNs derived from in vitro cultures can be used for cellular mechanistic studies as well as in vivo studies testing cell replacement strategies and modeling human disease in animal models, with the ultimate goal of replacing dysfunctional MNs in degenerative disorders like amyotrophic lateral sclerosis. To enhance MN differentiation and surmount some of these existing difficulties, Jianping Fu and colleagues (Sun et al., 2014) developed a mechanical platform that enhances differentiation efficiency of hPSCs into MNs by >4-fold and significantly shortens the time period required for MN maturation.

This platform capitalizes on advances using micromolded poly-(dimethylsiloxane) (PDMS) micropost arrays (PMAs) to culture cells (Fu et al., 2010). PDMS is widely used to construct microscale devices for cell culture and microfluidic applications (Fu et al., 2010). PDMS surfaces are continuous and thus cannot change rigidity without altering other characteristics of the material. In contrast, PMAs have a constant surface geometry whose rigidity is varied by adjusting heights of isolated microposts to cover the stiffness range exhibited by soft tissues without altering the contact area available for cell-matrix interactions or tethering of cell-surface integrins to

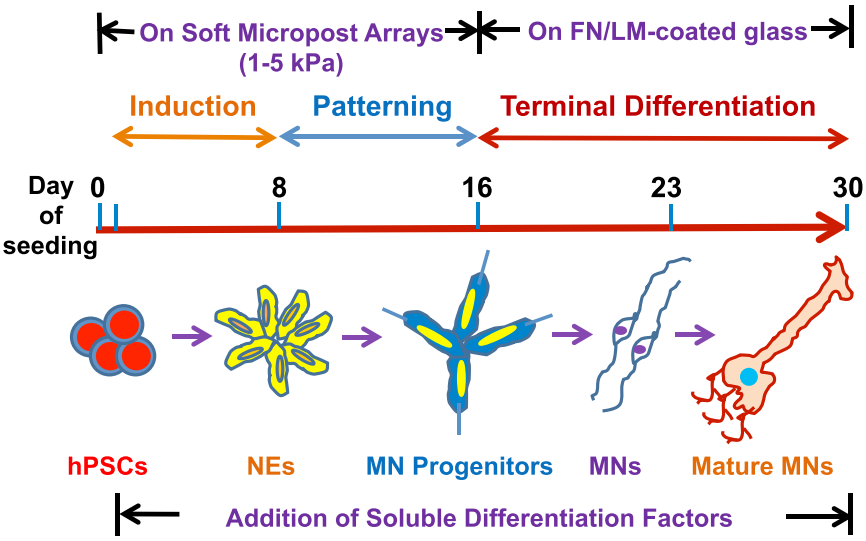

Figure 1. Schematic for Generating Motor Neurons from Human Pluripotent Stem Cells on PDMS-Based Micropost Arrays

hPSCs were seeded and maintained on vitronectin-coated soft micropost arrays before being transferred onto FN/LM-coated glass to facilitate induction and maturation of MNs. Different combinations of soluble signaling factors were added at the beginning of each stage (induction, patterning, or terminal maturation) of hPSC differentiation. NEs, neuroepithelial cells; FN, fibronectin; LM, laminin.

vitronectin-coated surfaces. The rigidity of soft tissues can range from 0.1 kPa to 100 kPa (Discher et al., 2009), in contrast to standard culture dishes with a rigidity of $>10^4$ kPa.

Sun et al. have harnessed the advantages of PMAs for directed differentiation of hPSCs into MNs (Figure 1). hPSCs were seeded on vitronectin-coated PMAs of varying stiffnesses in neuroepithelial (NE)-inducing culture and induction efficiency was assayed by expression of the neuroectodermal marker *PAX6*. After 8 days, the percentage of PAX6+ NE cells was 3-fold higher on soft PMAs (1–5 kPa) than on rigid PMAs (>14 kPa) or vitronectin-coated glass ($>10^4$ kPa). Cells were cultured for an additional 8 days with retinoic acid (RA) and Purmorphamine to induce caudalization and ventralization patterning cues, and cells induced on soft PMAs showed an 8-fold enrichment in expression of the MN progenitor marker *Olig2* as compared with cells maintained on stiffer PMAs. After transferring induced MN progenitors onto coverslips for 14 more days under conditions appropriate for MN maturation, cells from soft PMAs displayed a 13-fold enrichment in purity of mature MN subtypes over cells induced on stiff PMAs or glass. These MNs generated action potentials and expressed high levels of choline acetyltransferase, demonstrating derivation of mature MNs from hPSCs.

The authors next examined whether varying substrate rigidity influenced mechanotransduction during hPSC differentiation to enhance MN generation. Human mesenchymal stem cells (MSCs) differentiate toward different lineages in response to culturing on matrices of differing rigidity (Engler et al., 2006), and mouse ESCs differentiate in response to applied shear forces even in the presence of factors that maintain the pluripotent state, such as leukemia inhibitory factor (LIF) (Chowdhury et al., 2010). The YAP/TAZ pathway is required for MSC differentiation and transduces mechanical signals arising from matrix rigidity to the nucleus to coordinate transcriptional responses (Dupont et al., 2011). When Sun et al. compared the effects of culturing cells on soft PMAs with those of rigid PMAs, they found that phosphorylation of YAP was higher in the cytoplasm of the cells cultured on soft PMAs than that in cells cultured on rigid PMAs, with a corresponding decrease in nuclear YAP localization suggesting an inability of p-YAP to translocate into the nucleus. When in the nucleus, YAP regulates the activity of Smad transcription factors, and the authors found that YAP and p-Smad were colocalized only in the nuclei of cells cultured on rigid PMAs. Consistently, Smad phosphorylation was decreased in cells cultured on soft PMAs. The authors further demonstrated that the cytoskeleton regulates YAP localization in this system by showing that nuclear localization of YAP responded to an alteration in the actin cytoskeleton. Nuclear YAP localization was dramatically decreased by an inhibition of ROCK and could be enhanced by stimulation of RhoA, two enzymes that promote actin remodeling and stress fiber formation in response to mechanical cues, suggesting that tension-dependent mechanotransduction is important for the regulation of YAP translocation and signaling.

The findings of Fu and colleagues that MN differentiation is enhanced by YAP-dependent reductions in p-Smad levels are consistent with findings showing that YAP/TAZ-mediated p-Smad accumulation in the nucleus is necessary for maintaining hPSC pluripotency (Varelas et al., 2008). The mechanisms that underlie these effects, however, remain unresolved. For example, it is not clear how culturing cells on soft PMAs leads to downregulated phosphorylation of Smad proteins. Cells grown on soft PMAs exhibited few stress fibers, suggesting that altered actin dynamics may release Lats1, a kinase that phosphorylates YAP and prevents it from translocating into the nucleus. This is supported by the finding that stimulating RhoA activity using lysophosphatidic acid (LPA) completely blocked induction of $Pax6^+$ NE cells on soft PMAs, and siRNA-induced silencing of *Lats1* expression decreased the percentage of $Pax6^+$ cells on soft PMAs to the same level as that observed when culturing cells on rigid PMAs (Sun et al., 2014). Interestingly, Sun et al. find that culture on soft PMAs elevated expression of neural posterior markers *HOXB1*, *HOXB4*, and *HOXB8*, even in the absence of caudalizing RA, suggesting that local matrix compliance alone could alter the fate of anterior/posterior patterning. Furthermore, soft PMAs alone promoted much higher expression of *HOXB8* than RA, implying that local matrix compliance can be more potent than soluble factors in determining anterior/posterior patterning, consistent with recent findings that insoluble matrix softness is important in organization of germ layers in an in vitro mouse ESC culture (Poh et al., 2014).

The findings of Sun et al. (2014) demonstrating an improved protocol via substrate rigidity manipulation and the finding of crosstalk between YAP/TAZ and soft-matrix mediated mechanotransduction represent a significant step toward better understanding of MN differentiation from hPSCs. Another recent report describes high-efficiency MN differentiation from hPSCs with a shortened maturation duration by utilizing small molecules in combination with seeding cells on a combination of four matrix proteins (Qu et al., 2014), and it will be interesting to determine whether YAP and Smad signalings are altered under these conditions as well. The work of Fu and colleagues will stimulate further exploration and application of cell mechanics and mechanotransduction in stem cell biology and regenerative medicine.

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