Direct reprograming of mouse fibroblasts to cardiomyocytes using Yamanaka factors on engineered hydrogels

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Statement of Purpose: Direct reprogramming strategies are similar to iPS cell-sourcing strategies but bypass the pluripotent state, saving time and resources. Efe *et al.* (1) have directly reprogrammed mouse embryonic fibroblasts (MEFs) to cardiomyocytes (CMs, ~40% efficiency by FACS) on Matrigel-coated tissue culture polystyrene (TCPS), a stiff material that non-specifically adsorbs serum proteins. Poly (ethylene glycol) (PEG)-based materials can be used to present precise quantities of biologically active molecules at the desired mechanical stiffness, without binding non-specific proteins. By examining expression of cell adhesion receptor genes expressed during the reprograming process, we designed hydrogel surfaces that promote cell adhesion through laminin and vitronectin receptors. We demonstrated that direct reprogramming of fibroblasts to CMs is more efficient on engineered materials than on coated TCPS. This illustrates that stem cell interactions with biomaterials may be better tuned using engineered materials than with proteins adsorbed to TCPS.

Methods: MEFs containing doxycycline-inducible reprogramming factors (2) were plated on Matrigel-coated (11.4 μg/cm^2) 24-well TCPS plates as described (1) or laminin coated (11.4 μg/cm^2) 24-well TCPS plates. PEG-octavinylsulfone and PEG-octaamine hydrogels were incubated overnight with varying concentrations of RGD (0.4 mg/cm^2 = 1x) (3) and/or laminin (11.4 μg/cm2 = 1x) (3). The reprogramming protocol included induction of the reprogramming factors with doxycycline for 6 days, inhibition of pluripotency with the Jak/Stat inhibitor JI1 until day 9, and induction of cardiogenesis with BMP4 beginning at day 9. Numbers of beating patches were quantified by phase contrast microscopy. Cells were immunofluorescently stained for sarcomeric α-actinin and DAPI. Actinin to DAPI positive area ratios were quantified in Matlab (n=3 gels, ≥ 5 randomly selected images/gel). Values are presented as average ± standard deviation. IHC and beating patch data were analyzed by ANOVA with post-hoc Tukey-Kramer test.

Results: Examining gene expression data during the first three days of doxycycline treatment (4) revealed that laminin and laminin receptors were upregulated, while α_5 and α_v expression was not substantially decreased. This suggested to us that laminin would play a critical role in adhesion during reprograming, but that adhesion through RGD peptides may also be beneficial. We therefore used linear RGD peptides to target α_v integrins combined with laminin, both of which were attached to PEG hydrogels. Cells were seeded on the surfaces of the gels and assessed for cardiomyocyte differentiation at day 18. Numbers of beating patches on Matrigel-coated TCPS (240 ± 57 per 100,000 MEFs plated) were similar to what was described by Efe *et al.* (257 ± 17 per 100,000 MEFs plated or 17.2 ± 5.8/well). Interestingly, coating TCPS with laminin-1 instead of Matrigel yielded statistically similar results (17.3 ± 5.0/well), indicating that laminin may be a suitable substitute for Matrigel in the standard protocol, which is useful due to batch-to-batch variation in Matrigel. Numbers of clusters on PEG gels presenting RGD at 1x concentration were slightly lower than on TCPS with Matrigel or laminin, but increasing concentrations of adhesion molecules yielded greater numbers of beating patches (43 ± 12.4 for 5x laminin/5x RGD) as well as higher sarcomeric α-actinin positive areas (Fig. 1). Total numbers and projected areas of differentiated colonies were visibly affected by alterations in the concentrations of adhesion molecules (Fig. 1B). Thus, designed surfaces outperformed those obtained by simple adsorption on TCPS.

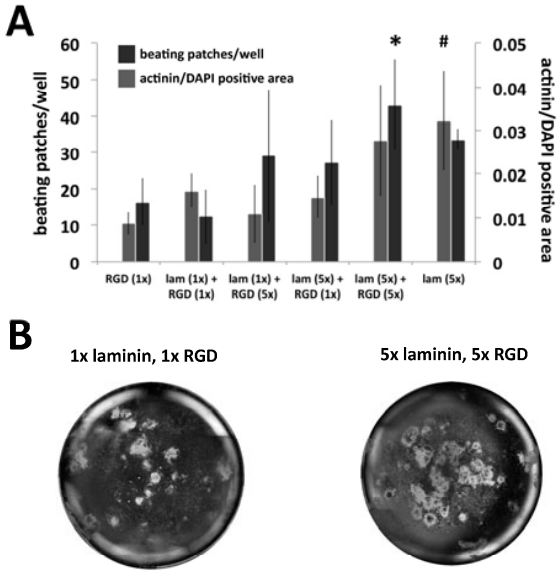

Figure 1: A) Increased concentrations of adhesion molecules on PEG gels resulted in greater numbers of beating patches (left axis) and α-actinin-positive cells (right axis). *p<0.05 versus 1x RGD and 1x RGD + 1x lam patch counts. # p<0.05 versus 1x RGD actinin/DAPI positive area. B) A noticeable dose dependency existed between adhesion molecule concentration and number of differentiated colonies, imaging the entire well of a 24-well plate.

Conclusions: Gene expression may guide the design of surfaces to promote direct reprograming of fibroblasts to cardiomyocytes, which is both faster and more efficient than producing iPS cells prior to cardiomyocyte differentiation.

References: 1) Efe *et al. Nat. Cell Bio.* 2011; 13: 215. **2)** Takahashi *et al. Cell.* 2006; 126: 663. **3)** Smith *et al. Acta Biomat.* 2012; 8: 31,**4)** Koche *et al*. *Cell Stem Cell.*2011, 8:96.