

Muscle-on-chip: An in vitro model for donor–host cardiomyocyte coupling

Pieterjan Dierickx and Linda W. Van Laake

Division of Heart and Lungs, Department of Cardiology, University Medical Center Utrecht and Hubrecht Institute, 3584 CX Utrecht, Netherlands

A key aspect of cardiac cell-based therapy is the proper integration of newly formed cardiomyocytes into the remnant myocardium after injury. In this issue, Aratyn-Schaus et al. (2016. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201508026>) describe an in vitro model for heterogeneous cardiomyocyte coupling in which force transmission between cells can be measured.

Isolation and subsequent differentiation of stem cells into cells of the cardiac lineage has improved significantly over the past years and is now performed with good efficiency (Dierickx et al., 2012). However, the maturation status of stem cell-derived cardiomyocytes (SC-CMs) is embryonic-like in comparison to in vivo cardiomyocytes (Synnergren et al., 2012). The use of cardiac progenitor cells and stem cell-derived cardiomyocytes for heart repair has nevertheless been explored in preclinical (Van Laake et al., 2007; Chong et al., 2014) and clinical (Bolli et al., 2011; Malliaras et al., 2014; Menasché et al., 2015) trials. The reported efficacy of these cardiac cell-based therapies is variable and predominantly linked to paracrine effects. Specifically, the contribution of donor cells to contractility appears rather limited. An assumed lack of electrical coupling between donor and host cells, resulting in suboptimal or nondurable improvement of cardiac function, is commonly regarded as the explanation for this phenomenon (Passier et al., 2008). In this issue, Aratyn-Schaus et al. provide a different perspective. Using an in vitro model of heterogeneous cell coupling, they observed that despite electrophysiological coupling and synchronous contraction of weak “donor” and strong “host” cardiomyocytes, focal adhesion-like structures at cell–cell junctions were associated with disrupted force transmission between the cells.

The formation of intercalated discs, specialized cell–cell junctions consisting of desmosomes and gap junctions that transmit electrochemical signals and mechanical forces, is essential for synchronized contraction between cardiomyocytes. Their establishment relies on interactions between cells and the extracellular matrix and a redistribution of cell–cell adhesions, which is further linked to the contractility status of the cells. Because newly differentiated cardiomyocytes are less mature than their endogenous counterparts, nonisometric force generation and hampered transmission may occur between the two cell types. As measuring cellular traction forces of coupled myocytes in vivo is intrinsically difficult, Aratyn-Schaus et al. (2016) developed an in vitro system to measure mechanical coupling of cardiomyocytes and to study how force/stress

is transmitted from cell to cell. The authors use microtissues (μ tissues) consisting of an immature cell type (mouse SC-CMs) and a more mature cell type (mouse neonatal cardiomyocyte [nCM]) to simulate the therapeutic setting of new and native cells, respectively. To achieve this, microscopic fibronectin islands were printed on a soft gel to support the plating of a mixture of nCMs and SC-CMs, thereby generating a μ tissue consisting of the two cell types. Both murine embryonic and induced pluripotent stem cells (mES and miPS cells) were used for SC-CM differentiation.

Before analyzing their μ tissues, Aratyn-Schaus et al. (2016) characterized the cell types in terms of cytoskeleton organization and contractile force. All cells showed striated myofibrils extending parallel to the longitudinal axis of the cells, with highly aligned actin networks. The researchers compared the contractile force of these spontaneously beating cells via traction force microscopy, and they showed the localization of stress at the proximal edges of the cells during contraction, which could be pinpointed by large longitudinal force vectors (Fig. 1 A). Even though SC-CMs had normal striated myofibrils, their peak systolic force was two times lower than that of nCMs. Thus, despite a similar structural organization, SC-CMs are weaker than nCMs.

Actual cardiac regeneration requires that new cells interact with the native myocardium via the formation of intercalated discs that contain gap junctions. Staining for the junction proteins involved in electrical and mechanical coupling, connexin-43 and β -catenin, showed cell–cell junction formation in both homogeneous and heterogeneous (mES-CM/nCM and miPS-CM/nCM) μ tissues, suggesting that the described chip-based platform represents the best-case scenario of in vivo engraftment. The authors also checked the electrochemical coupling of their μ tissues using dual-excitation ratiometric analysis to quantify Ca^{2+} transients. Despite synchronized activity in each type of pair, homogeneous pairs of nCMs showed steeper Ca^{2+} fluxes than heterogeneous pairs. Additionally, lower levels of diastolic calcium were reported in homogeneous SC-CM couples than in homogeneous nCM couples. Strikingly, when neonate myocytes were coupled to SC-CMs, their diastolic Ca^{2+} levels declined by 30% as compared with homogeneous neonate pairs, implying a negative effect of the immature cell on the more mature cell. Immature cardiomyocytes are thus able to couple with mature cardiomyocytes in synchronized μ tissues, but differences in calcium handling may hamper contractility.

© 2016 Dierickx and Van Laake This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>).

Correspondence to Linda W. Van Laake: l.w.vanlaake@umcutrecht.nl

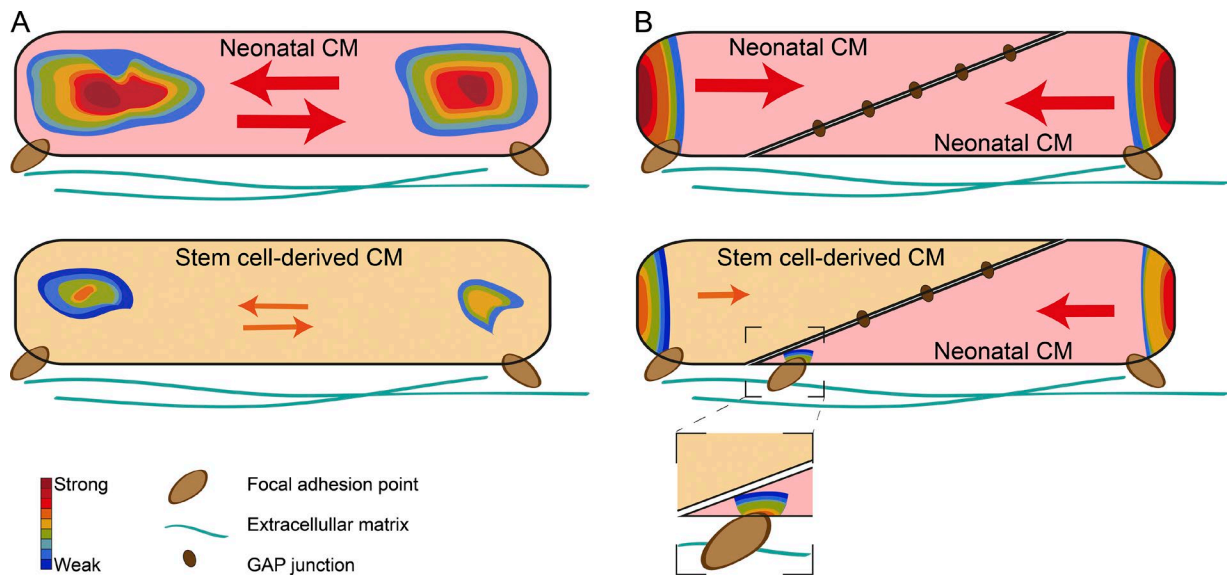


Figure 1. **In vitro cardiomyocyte coupling model.** (A) Neonatal cardiomyocytes (CMs) show greater contraction forces (arrows) than SC-CMs. Force generation at the lateral ends of the cells is depicted as a heat map. (B) Coupling of an immature SC-CM with a stronger nCM (bottom) results in extra focal adhesions (inset) compared with the homogeneous coupling of nCMs (top).

Aratyn-Schaus et al. (2016) next used traction force microscopy to study whether the difference in intrinsic force between SC-CMs and nCMs affects the function of μ tissues. Indeed, compared with neonate couples, the amount of traction stress and substrate displacement during contraction was far lower in heterogeneous SC-CM/nCMs couples (Fig. 1 B). Interestingly, an additional traction stress point was observed in heterogeneous pairs near cell–cell junctions (Fig. 1 B). Next to the focal adhesions at the lateral ends of all μ tissues, this new point of traction was shown to accompany extra focal adhesions as observed by vinculin staining. A possible explanation for such a phenomenon of extra traction stress points joined by newly formed focal adhesions could be the prevention of mechanical disruption of the newly formed junctions between the two cells.

To unravel whether coupled cells of different origins show mechanical synchrony, Aratyn-Schaus et al. (2016) measured the longitudinal traction forces exerted by the cells on the substrate. All contracted in synchrony and, as expected, homogeneous nCM pairs produced higher traction forces than SC-CM pairs. In contrast, the difference in force between cells within a heterogeneous SC-CM/nCM couple was >30% of the total systolic force produced by the pair. Dissipating the surplus of force may be required to keep the cells together. Further, to test whether the model displays features of known disease-related phenotypes, the dimensions of the fibronectin islands on which cells are plated were adjusted to mimic a hypertrophic morphology or their stiffness was modified to simulate a fibrotic environment. In both cases, traction forces at the junctions of the two cells on the substrate were increased. These results all support the muscle-on-chip model as a promising tool to study myocardial disease in vitro.

Because there are important differences in the transcriptional and functional mechanisms underlying the maturation of primary cardiomyocytes and SC-CMs in various species (Sheehy et al., 2014), Aratyn-Schaus et al. (2016) developed an unbiased generalized computational method to analyze μ tissue mechanics. In this model, in which the contractile behavior of two cells is seen as a 2D continuum, a positive correlation between

adhesion and traction stress was added. For heterogeneous tissues, smaller isometric tension values were accounted for and strain rate constants for the SC-CMs were based on known inotropic effects of different calcium fluxes (Bers, 2001). In their simulations, the authors noted high traction stress solely at the lateral sides of the cardiomyocytes in homogeneous μ tissues, whereas in heterogeneous μ tissues an additional traction stress point emerged near the tip of the cell–cell junction, which overall is in line with the experimental data (Fig. 1 B). Simulating cellular stress and the average shortening of both cells also confirmed that stem cell–derived myocytes shortened less than neonate myocytes and that none of the cells lengthened, as observed in vitro. Altogether, this computational analysis suggests that coupling of two cells with uneven contractile properties such as isometric tension is sufficient to generate force transmission remodeling at the junction between cells.

In conclusion, Aratyn-Schaus et al. (2016) developed a muscle-on-chip in vitro system to model integration and coupling of new cells with native cells in the heart. They discovered that SC-CMs are able to align and couple with nCMs, form gap junctions, and contract synchronously, regardless of their intrinsic weaker contractile properties. The stronger neonatal cells seem to sense the force imbalance and react by dissipating the excess of energy to the substrate through the formation of additional focal adhesion points. This finding offers a possible explanation as to how, until now, only modest long-term beneficial contractile effects have been observed in preclinical trials, even when using bona fide cardiomyocytes.

Even though the computational model supports the translational potential of the murine muscle-on-chip assay, the findings of Aratyn-Schaus et al. (2016) should eventually be reproduced using human adult and SC-CMs. If similar in human, the results would highlight the importance of fully characterizing newly introduced therapeutic cardiomyocytes to prevent immature or otherwise weaker donor cells from exerting a negative effect on the function of spared recipient cardiomyocytes. From the standpoint of mechanical force generation described here, it might be beneficial to strive for maximal in vitro donor

cell maturation before transplantation. Several maturation-enhancing differentiation protocols have been developed recently, especially for human pluripotent SC-CMs (Birket et al., 2015; Ribeiro et al., 2015). Another consideration, however, is that terminally matured adult-like cardiomyocytes are much less likely to survive transplantation compared with immature fetal-like cardiomyocytes (Reinecke et al., 1999). The final maturation steps may therefore better occur in vivo after transplantation, and it is possible that the environment of an injured heart supports such development by itself or with additional measures (Laflamme et al., 2007; Van Laake et al., 2007).

Because in vivo cell coupling analysis and force transmission measurements are intrinsically difficult, the in vitro muscle-on-chip model by Aratyn-Schaus et al. (2016) represents an important step forward toward unraveling the complex molecular mechanisms underlying the integration of newly formed cells to an existing tissue. The model could be further developed to simulate disease states in vitro by adding supportive/interactive cells such as fibroblasts and endothelial cells and by using different substrates as well as defected cardiomyocytes. Additionally, cardiac cells with different maturation states could be tested in the future, as it is critical to determine the optimal cell type and state for cardiac cell-based therapy.

Acknowledgments

The authors are grateful to Marit W. Vermunt for the help with the figure.

This work was supported by ZonMw (Veni 91612147) and the Netherlands Heart Foundation (2013T056).

The authors declare no competing financial interests.

Submitted: 21 January 2016

Accepted: 26 January 2016

References

- Aratyn-Schaus, Y., F.S. Pasqualini, H. Yuan, M.L. McCain, G.J.C. Ye, S.P. Sheehy, P.H. Campbell, and K.K. Parker. 2016. Coupling primary and stem cell-derived cardiomyocytes in an in vitro model of cardiac cell therapy. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201508026>
- Bers, D.M. 2001. Excitation-Contraction Coupling and Cardiac Contractile Force. Springer Netherlands, Dordrecht, Netherlands. 237 pp. <http://dx.doi.org/10.1007/978-94-010-0658-3>
- Birket, M.J., M.C. Ribeiro, G. Kosmidis, D. Ward, A.R. Leitoguinho, V. van de Pol, C. Dambrot, H.D. Devalla, R.P. Davis, P.G. Mastroberardino, et al. 2015. Contractile defect caused by mutation in MYBPC3 revealed under conditions optimized for human PSC-cardiomyocyte function. *Cell Reports.* 13:733–745. <http://dx.doi.org/10.1016/j.celrep.2015.09.025>
- Bolli, R., A.R. Chugh, D. D'Amario, J.H. Loughran, M.F. Stoddard, S. Ikram, G.M. Beache, S.G. Wagner, A. Leri, T. Hosoda, et al. 2011. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet.* 378:1847–1857. [http://dx.doi.org/10.1016/S0140-6736\(11\)61590-0](http://dx.doi.org/10.1016/S0140-6736(11)61590-0)
- Chong, J.J.H., X. Yang, C.W. Don, E. Minami, Y.-W. Liu, J.J. Weyers, W.M. Mahoney, B. Van Biber, S.M. Cook, N.J. Palpant, et al. 2014. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature.* 510:273–277. <http://dx.doi.org/10.1038/nature13233>
- Dierickx, P., P.A. Doevendans, N. Geijsen, and L.W. van Laake. 2012. Embryonic template-based generation and purification of pluripotent stem cell-derived cardiomyocytes for heart repair. *J. Cardiovasc. Transl. Res.* 5:566–580. <http://dx.doi.org/10.1007/s12265-012-9391-6>
- Laflamme, M.A., K.Y. Chen, A.V. Naumova, V. Muskheili, J.A. Fugate, S.K. Dupras, H. Reinecke, C. Xu, M. Hassanipour, S. Police, et al. 2007. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat. Biotechnol.* 25:1015–1024. <http://dx.doi.org/10.1038/nbt1327>
- Malliaras, K., R.R. Makkar, R.R. Smith, K. Cheng, E. Wu, R.O. Bonow, L. Marbán, A. Mendizabal, E. Cingolani, P.V. Johnston, et al. 2014. Intracoronary cardiosphere-derived cells after myocardial infarction: evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricular dysfunction). *J. Am. Coll. Cardiol.* 63:110–122. <http://dx.doi.org/10.1016/j.jacc.2013.08.724>
- Menasché, P., V. Vanneaux, A. Haggège, A. Bel, B. Cholley, I. Cacciapuoti, A. Parouchev, N. Benhamouda, G. Tachdjian, L. Tosca, et al. 2015. Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report. *Eur. Heart J.* 36:2011–2017. <http://dx.doi.org/10.1093/eurheartj/ehv189>
- Passier, R., L.W. van Laake, and C.L. Mummery. 2008. Stem-cell-based therapy and lessons from the heart. *Nature.* 453:322–329. <http://dx.doi.org/10.1038/nature07040>
- Reinecke, H., M. Zhang, T. Bartosek, and C.E. Murry. 1999. Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts. *Circulation.* 100:193–202. <http://dx.doi.org/10.1161/01.CIR.100.2.193>
- Ribeiro, M.C., L.G. Tertoolen, J.A. Guadix, M. Bellin, G. Kosmidis, C. D'Aniello, J. Monshouwer-Kloots, M.J. Goumans, Y.-L. Wang, A.W. Feinberg, et al. 2015. Functional maturation of human pluripotent stem cell derived cardiomyocytes in vitro—correlation between contraction force and electrophysiology. *Biomaterials.* 51:138–150. <http://dx.doi.org/10.1016/j.biomaterials.2015.01.067>
- Sheehy, S.P., F. Pasqualini, A. Grosberg, S.J. Park, Y. Aratyn-Schaus, and K.K. Parker. 2014. Quality metrics for stem cell-derived cardiac myocytes. *Stem Cell Rep.* 2:282–294. <http://dx.doi.org/10.1016/j.stemcr.2014.01.015>
- Synnergren, J., C. Améen, A. Jansson, and P. Sartipy. 2012. Global transcriptional profiling reveals similarities and differences between human stem cell-derived cardiomyocyte clusters and heart tissue. *Physiol. Genomics.* 44:245–258. <http://dx.doi.org/10.1152/physiolgenomics.00118.2011>
- Van Laake, L.W., R. Passier, J. Monshouwer-Kloots, A.J. Verkleij, D.J. Lips, C. Freund, K. den Ouden, D. Ward-van Oostwaard, J. Korving, L.G. Tertoolen, et al. 2007. Human embryonic stem cell-derived cardiomyocytes survive and mature in the mouse heart and transiently improve function after myocardial infarction. *Stem Cell Res. (Amst.)* 1:9–24. <http://dx.doi.org/10.1016/j.scr.2007.06.001>