Cardiac stem cell mechanosome and mechanosensitivity develop with differentiation

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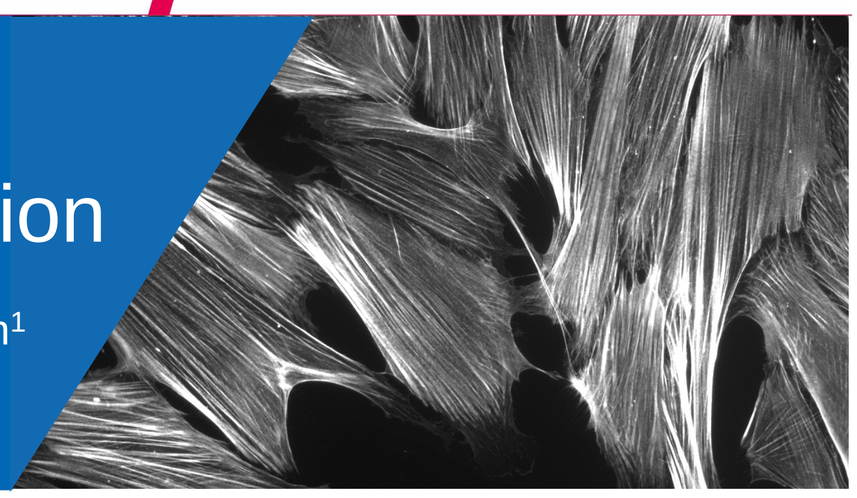

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Introduction

Stem cell mechanosensing and mechanoresponse is crucial for tissue regeneration in mechanically functional organs like the heart. However, very little is known about how cardiac resident stem cells, such as cardiomyocyte progenitor cells (CMPCs), sense and respond to the mechanical stimuli provided by the beating heart. Thus, the complex of cellular structures relevant for mechanosensing, made of focal adhesions (FAs) and actin stress fibers, the 'mechanosome', needs to be explored. Identifying the key players and the underlying mechanisms of cardiac stem cell mechanotransduction is essential to achieve optimal migration and integration of endogenous cardiac cells into the injured site. Here we demonstrate that the mechanosensitivity of CMPCs relies on the mechanosome, which develops with early cardiac differentiation.

Materials and methods

Cardiomyogenic differentiation of human L9TB CMPCs was induced by biochemical stimulation for 14 days (d_{diff} 14, predifferentiated CMPCs) [1]. Uniaxial cyclic strain (10% at 0.5Hz) was applied for 48h (day0-day2) to undifferentiated (undiff) and predifferentiated (prediff) CMPCs seeded on collagen IV-coated Bioflex membranes. Unstrained samples were used as control.

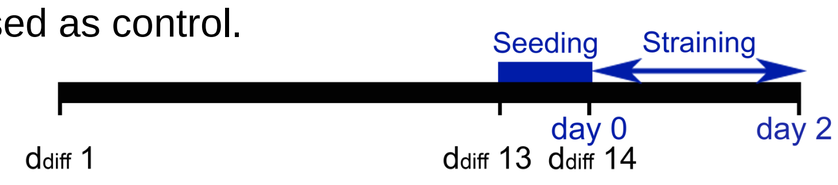

Figure 1: Timeline of the experiment.

CMPCs respond to mechanical cues upon differentiation

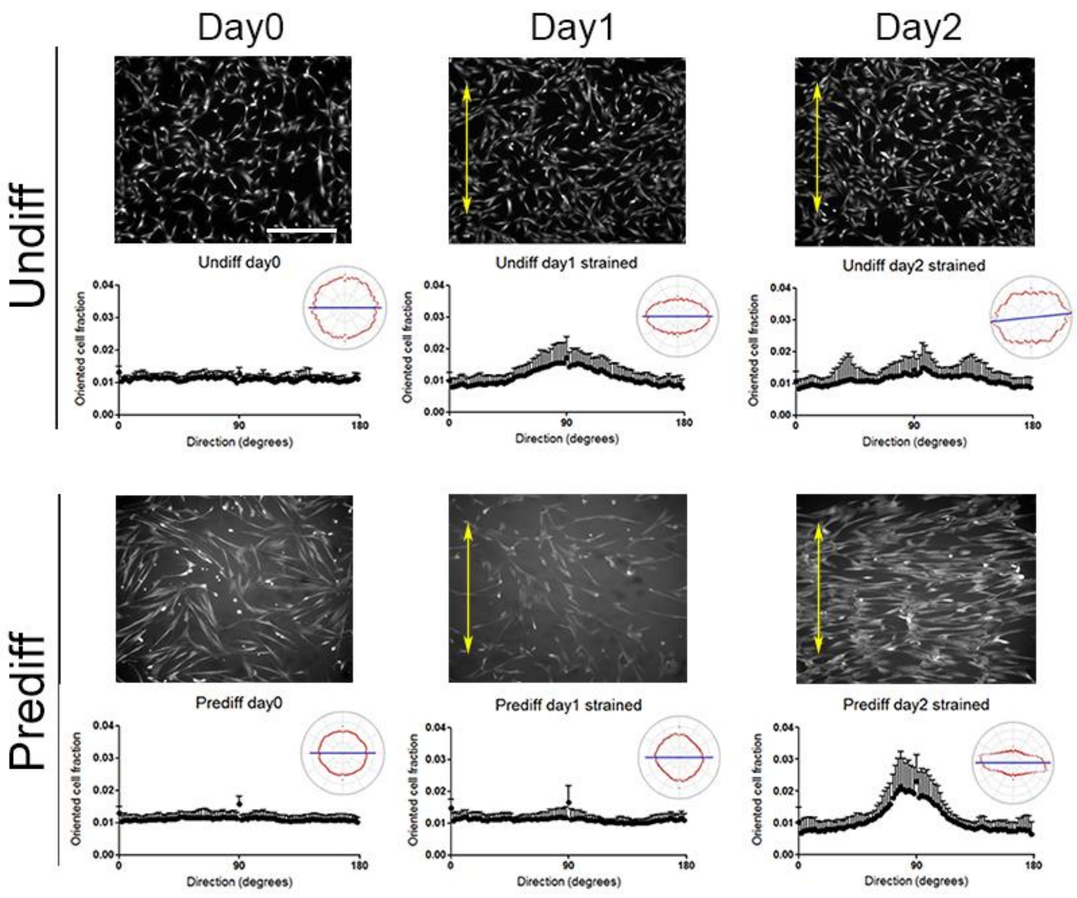

Figure 2: Mechanosensitivity is developed with differentiation in CMPCs. Upon cyclic strain, undifferentiated CMPCs (undiff) kept a random orientation, whereas predifferentiated CMPCs (prediff) reoriented perpendicularly to the direction of the strain after 2 days. Representative fluorescent images (calcein), frequency distribution and polar plots display the cell orientation distribution corresponding to each group. Scale bar indicates 400μm. Results are expressed as mean ± SD (N=16-23).

The CMPC mechanosome develops with differentiation and not with strain

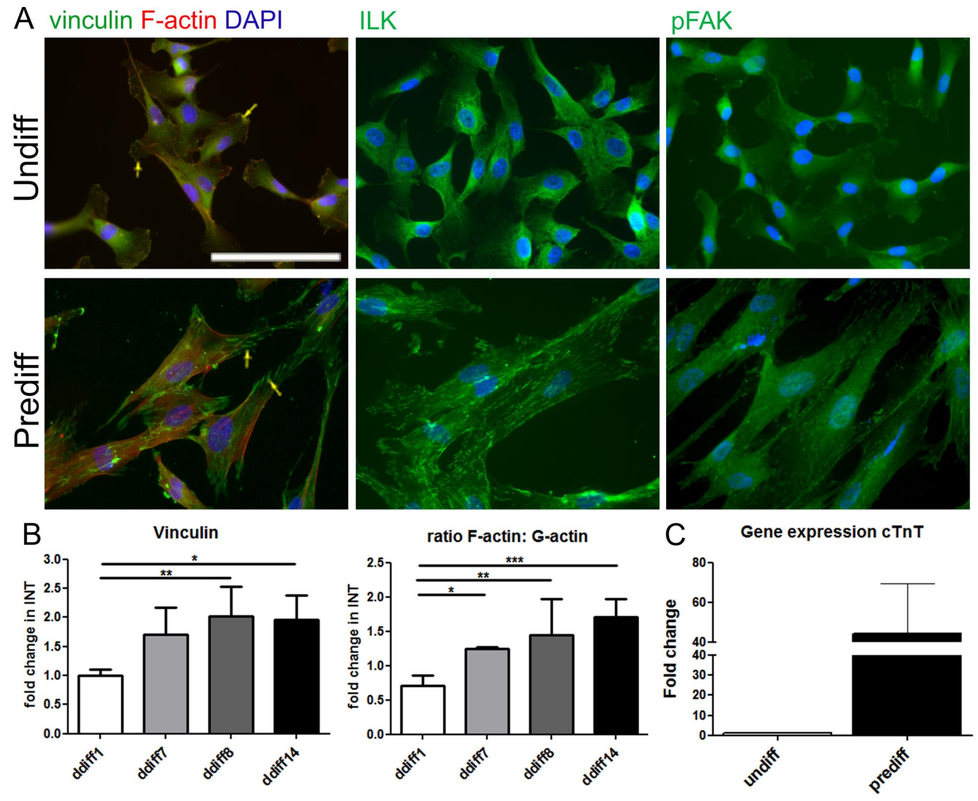

Figure 3: CMPCs develop the mechanosome upon early cardiac differentiation. A) Immunofluorescence images show the different organization of vinculin, integrin-linked kinase (ILK), and phosphorylated focal adhesion kinase (pFAK) in undifferentiated (undiff) and predifferentiated (prediff) CMPCs 24 hrs after seeding and before strain application (day0). B) Increased protein levels of vinculin and F:G-actin ratio indicate development of the mechanosome during CMPC early differentiation. C) Upregulation of the cardiomyocyte marker cardiac troponin T (cTnT) demonstrates the early cardiac differentiation of prediff CMPCs. Results are expressed as mean±SD (N=3). Scale bar 100μm.

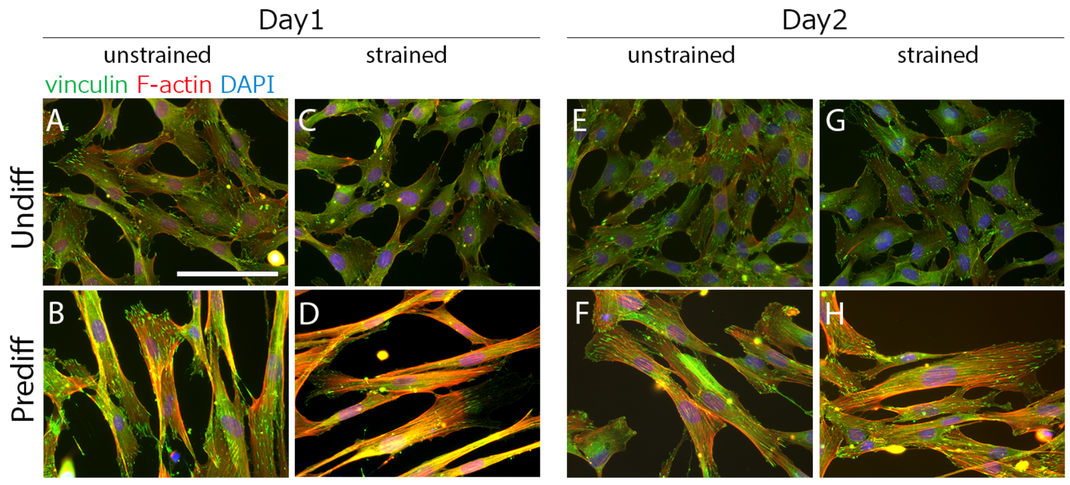

Figure 4: The mechanosome development is not induced by external mechanical stimuli. Immunofluorescence stainings of vinculin (green) and F-actin (red) at day1 and day2 of straining, did not show differences between unstrained and strained samples in both undifferentiated (undiff) and predifferentiated (prediff) CMPCs. Results are expressed as mean ± SD (N=3).

Conclusions and outlook

In conclusion, we showed for the first time that the CMPC mechanosensing apparatus in direct contact with the ECM, the mechanosome, only develops upon differentiation, and allows differentiating CMPCs to align in response to uniaxial strain. This suggests that the lack of a developed mechanosome in undifferentiated CMPCs shields them from the mechanical environment of the beating heart, whereas differentiating cells can sense and respond to cyclic strain by aligning.

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References:
1: Smits AM et al., Nature Protocols 2009;4(2):232-243