Production of human engineered heart tissue from induced pluripotent stem cells under cGMP-grade conditions for cardiac repair

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GMP-grade manufacturing process of engineered heart tissue (EHT) for transplantation

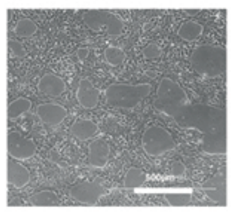
hiPSC expansion	*Cardiac Differentiation*	*EHT generation & maturation*	*Cryo-injury & Transplantation*	*Cardiac repair*	*Analysis*
14 days	*18 days*	*21 days*	*7 days*	*35 days*	*~ 100 days in total*

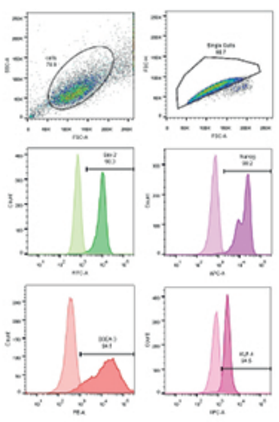
hiPSC expansion and cardiac differentiation

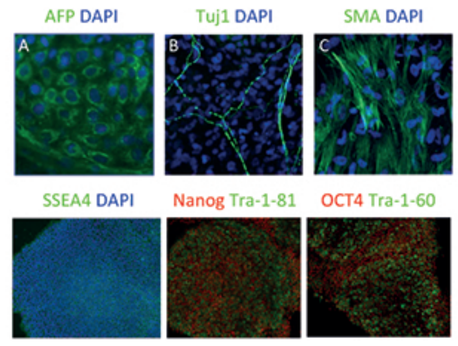
To be able to use the generated cardiomyocytes and EHTs for pre-clinical or clinical applications the human induced pluripotent stem cell (hiPSC) source is a cGMP-manufactured cell line (generated by Lonza Inc. / distributed by NIH). All steps of the hiPSC expansion and differentiation protocol need to be converted from research-grade to GMP-grade culture settings.

Criteria and conditions for hiPSC culture include:

- Defining culture criteria for GMP-grade hiPSC propagation, differentiation and storage
  → Defined and GMP-compatible cell adhesion proteins, cell detachment & dissociation reagents, serum-replacement
  cocktails, lipid sources, growth factors and inhibitors, cryo-media.
- Generation of master- and working- cell banks (MCB, WCB) of hiPSC and cell banks of differentiated cardiomyocytes
- Quality criteria for frozen cell stocks
  → Healthy karyotype, pluripotency, hiPSC markers and cardiac markers respectively, sterility, viability.



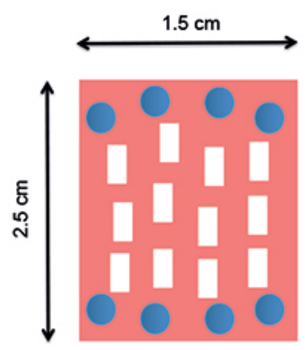




Baghbaderani, B. A., Tian, X., Neo, B. H., Burkall, A., Dimezzo, T., Sierra, G., et al. (2015). cGMP-Manufactured Human Induced Pluripotent Stem Cells Are Available for Pre-clinical and Clinical Applications. *Stem Cell Reports*, *5*(4), 647–659.

EHT generation and maturation

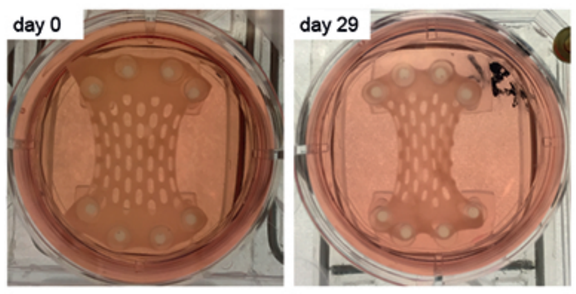
EHT Patch-Design

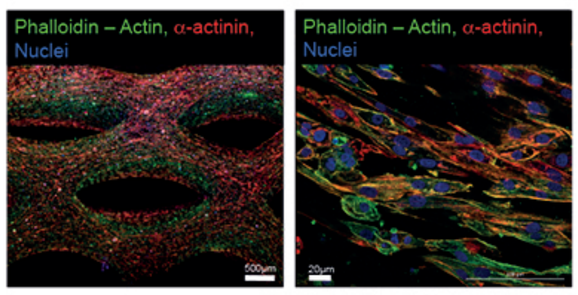


The EHT geometry was designed in patch-shape to better allow for a complete coverage of the scar tissue after cardiac injury.
The holes in the tissue patch create multiple force-lines for improved alignment of cardiomyocytes along the contraction axis and allow for a better supply with nutrients.

Static culture

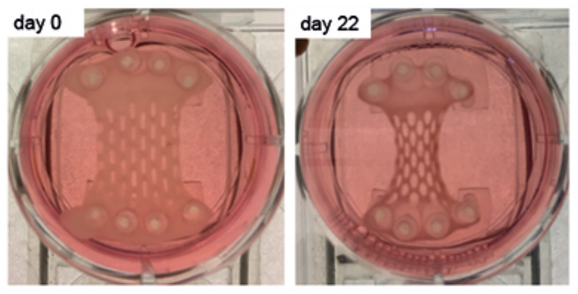
Serum-free medium

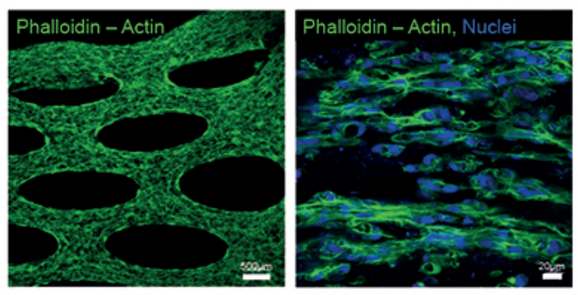




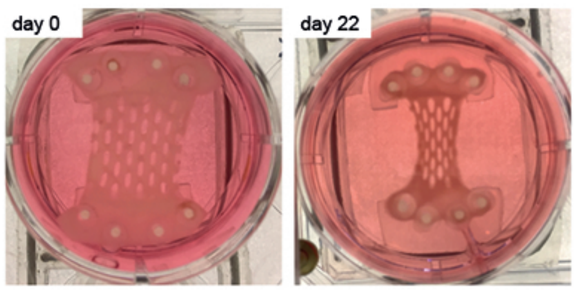
Dynamic culture

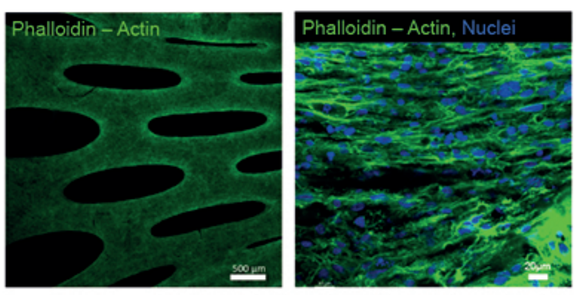
Serum-free medium





Serum-containing medium





The presented EHTs were generated from frozen stocks of hiPSC-differentiated cardiomyocytes (with a purity of above 80% cardiac troponin T positive cells) and contain 17 x 10^6 cells per 1700µl of a fibrinogen containing master mix. EHTs were cultured on a static (non-moving) or dynamic (rocking, ± 30° tilt, 0.4 Hz) platform for a period of minimum 21 days. The serum-free medium contained B27 serum-replacement and recombinant growth factors (IGF, FGF, VEGF, TGF-ß) in comparison to horse-serum supplemented medium without growth factors.

Under static conditions serum-free EHTs did not show robust contraction force, and cellular vitality within the tissue was lower compared to well-contracting serum-free EHTs under dynamic culture. Dynamic culture conditions support EHT remodeling and cellular compaction of the tissue as well as improved alignment of cardiomyocytes along the contraction axis.

Serum-containing EHTs showed robust muscle contraction and cell vitality under dynamic culture. However, under static culture conditions reduced cell alignment and compaction was also present (data not shown).

In order to generate and culture human EHTs under GMP-grade / xeno-free culture conditions following main components need to be addressed:

- Design of optimal EHT geometries based on infarct area and transplantation requirements
- Defining xeno-free EHT culture medium
  - Xeno-free support matrix (fibrinogen, other matrix components)
  - Serum-replacement or human serum
  - Recombinant growth factors of GMP-grade
  - Xeno-free recombinant protease-inhibitors for EHT stabilisation
- Defining culture conditions (static vs. dynamic) and culture duration for optimal maturation

EHT quality criteria for clearance for transplantation include:

- Intact muscle tissue patches with good remodeling morphology
- Contracting muscle tissue (defined force values are collected prior to transplantation)

Reinsch, M., & Weinberger, F. (2017). Stammzellbasierte kardiale Regeneration nach Infarkt. *Herz*, *43*(2), 109–114.
Weinberger, F., Breckwoldt, K., Pecha, S., Kelly, A., Geertz, B., Starbatty, J., et al. (2016). Cardiac repair in guinea pigs with human engineered heart tissue from induced pluripotent stem cells. *Science Translational Medicine*, *8*(363), 363ra148–363ra148.