FRIEDRICH-ALEXANDER UNIVERSITÄT ERLANGEN-NÜRNBERG
FACULTY OF MEDICINE

Universitätsklinikum Erlangen

Direct 3D printing of hiPSC-cardiomyocytes in collagen-based bioinks

Tilman U. Esser, Felix B. Engel – Department of Nephropathology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

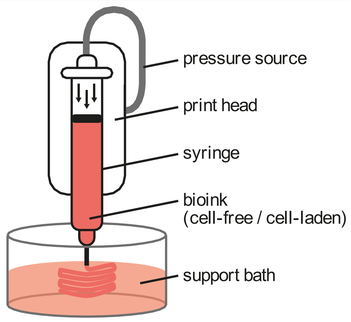
Purpose

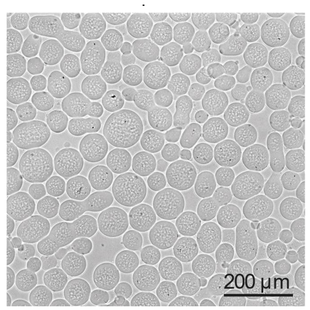
Artificial cardiac tissues are produced by combining cells of the heart with a biomaterial matrix. Generally, molds are used to cast tissues of a desired shape/geometry. 3D bioprinting allows for the generation of tissues with more complex geometries, as well as hierarchical and anisotropic structure. Previous studies utilizing bioprinting to produce artificial cardiac tissues showed limited functionality or required an additional structural “bioink” to produce stable constructs.

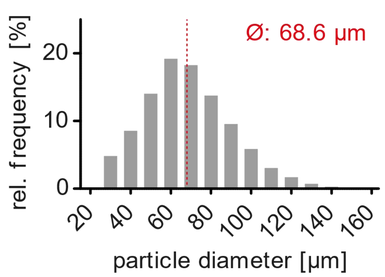
The aim of this project was to develop an approach which enables direct printing of hiPSC-cardiomyocytes into stable structures and allows for the formation of functional tissue.

Methods

An “in-gel” printing approach is employed, where the bioink is deposited into support bath consisting of gelatin / gum arabic microparticles. Microparticles are produced by complex coacervation and compacted by centrifugation. Support bath is melted away at 37°C after bioink gelation.



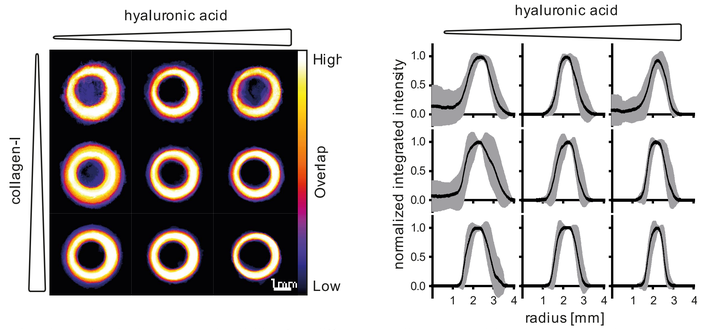




▲ Printing procedure and support bath. **Top right:** Schematic of printing setup for cell-free or cell-laden bioinks. **Bottom left:** Overview image of gelatin/gum arabic microparticles. **Bottom right**: Microparticle size distribution. Red dotted line indicates mean particle size.

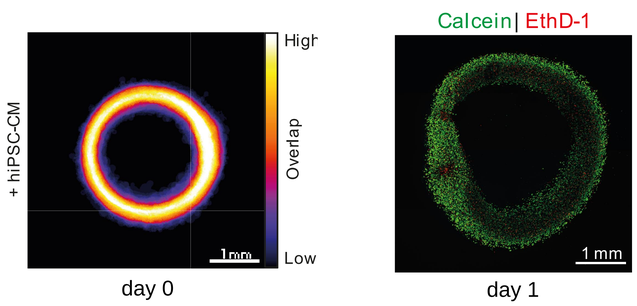
Results

Stable rings (5 x 5 x 1 mm) can be printed using collagen-I/hyaluronic acid bioinks. Formulations containing higher concentrations of collagen-I and hyaluronic acid show improved printing reproducibility.



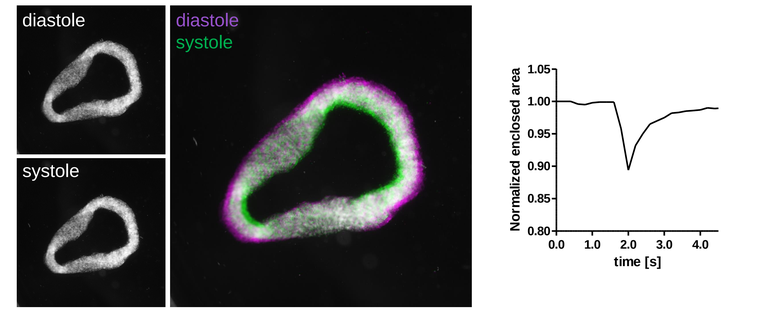
▲Evaluation of printing reproducibility for different collagen and hyaluronic acid concentrations. **Left: “**Overlap maps” of multiple printed rings (n=14-23). **Right:** Radial intensity profiles of printed rings. Mean (black lines) ± SD (grey area).

Bioinks containing hiPSC-derived cardiomyocytes remain printable with high reproducibility. Cells survive the printing process and show high viability. Printed rings develop spontaneous contractions as early as 3 days after fabrication. Printed rings were cultured under free-floating conditions for up to 30 days. Contractions became more synchronous. Immunofluorescence staining showed expression of cardiac Troponin I throughout the ring, albeit with a clear gradient from outside to inside.

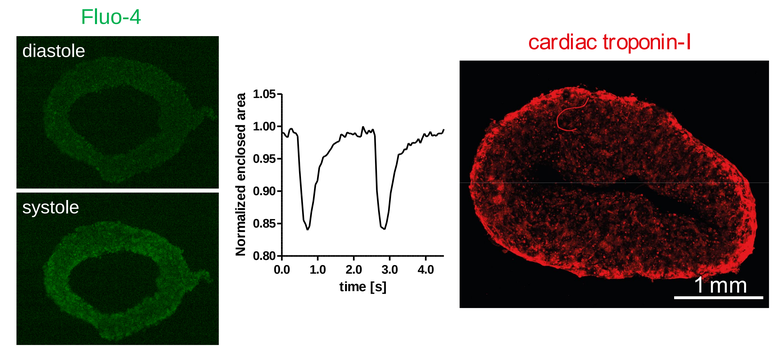


▲ Cell compatibility of printing approach. **Left:** “Overlap map” of multiple printed rings using a bioink containing hiPSC-derived cardiomyocytes (n=45). **Right:** Live/Dead staining of printed ring containing hiPSC-derived cardiomyocytes.

Results



▲ Spontaneous contractions of printed rings after 3 days. **Left:** Individual frames from videos of printed rings representing either non-contracted (diastole) or contracted (systole) state. **Middle:** Overlay of diastole and systole, illustrating change in displacement. **Right:** Area enclosed within ring over the course of a contraction cycle. Normalized to baseline.



▲ Printed rings cultured for 30 days. **Left:** Individual frames from videos of printed rings stained with calcium-sensitive dye Fluo-4. **Middle:** Area enclosed within ring over the course of two contraction cycles. Normalized to baseline. **Right:** Immunofluorescence staining for sarcomeric protein cardiac troponin-I.

Conclusion

This approach uses a two-component bioink to enable the direct printing of hiPSC-derived cardiomyocytes into stable constructs and allows for the formation of functional tissue.