Cardiac Surgery and Engineering

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3D Engineered tissues as angiogenic therapeutic approach and as functional cardiac models

Research Summary: The ultimate goal of the research group is to investigate angiogenic therapies based on engineered tissues (patches) to induce safe and efficacious angiogenesis in a chronic ischemic myocardium. For this purpose two different strategies are currently investigated relying on the use of either (1) VEGF-expressing cells or (2) heterogeneous cell population with a high angiogenic potential. The group also aims to (3) develop *in vitro* functional cardiac models to investigate processes of myocardial repair/regeneration. Research is funded by the Swiss National Science Foundation and Swiss Heart Foundation.

Patches engineered by VEGF-expressing cells. Therapeutic angiogenesis induced by exogenous VEGF delivery is a promising strategy. However, VEGF release at the microenvironmental level needs to be controlled to induce only normal capillary network, avoiding growth of aberrancies. A FACS-based technique was used to purify transduced human adipose tissue-derived mesenchymal stromal cells (ASC) that homogeneously express a safe VEGF dose from a heterogeneous primary population (Helmrich U, 2011) (collaboration with A. Banfi). Direct intramyocardial injections of ASC expressing safe VEGF-levels induced controlled angiogenesis in the heart, beside the poor cell survival observed (Melly and Marsano, 2012). Thereafter, skeletal myoblasts expressing safe VEGF levels co-cultured with cardiomyocytes in 3D scaffolds were shown to induce an efficacious angiogenesis in engineered cardiac tissues and superior cell survival upon implantation into an ischemic myocardium (Marsano, 2013). We then hypothesized that patches generated by controlled VEGF-expressing ASC could induce normal and efficient angiogenesis not only in the patch itself but also in the surrounding area, working as a controlled delivery system. We found that VEGF release induced normal angiogenesis in the patch already after 7 days, and in the surrounding avascular area (simulated by an empty 7mm-thick cell-free collagen sponge) after 28 days upon implantation in a subcutaneous rat model (Fig. 1). Patch prompt vascularization resulted in an increased survival of implanted cells up to 28 days (Boccardo and Gaudiello, 2016).

Patches engineered by cells with a high angiogenic potential. In this approach, we used human adipose tissue-derived stromal vascular fraction (SVF) cells as a heterogeneous cell population with a high angiogenic potential thanks to the presence of numerous endothelial/mural progenitors (collaboration with A. Scherberich). We hypothesized that perfusion-based bioreactor culture supported the maintenance of endothelial/mural cells as compared to static culture, thereby accelerating the whole construct vascularization and supporting the cell survival upon implantation in a subcutaneous rat model. Our findings showed that perfusion-based culture significantly modulated the initial SVF cell population composition, leading to a significant enrichment of the pericytes compared to static condition. The enriched perfusion-based engineered constructs showed an accelerated *in vivo* vessel ingrowth at 3 days and promoted the formation of blood vessels by cells of human origin.

3D functional cardiac models. Our angiogenic engineered tissues might also affect cardiac repair/regeneration by influencing cardiomyocyte maturation and functionality and progenitor cell recruitment. Therefore, we aimed here to generate 3D functional cardiac models as tool to investigate interactions of VEGF-expressing ASC/SVF cells and cardiomyocytes. We hypothesized that the recapitulation of the proper physiological conditions, mimicking the native tissue environment, enhanced the cardiomyocyte maturation, 3D organization and functionality. Culture medium perfusion systems were employed to mimic the highly dense capillary network present in the myocardium to ensure the cardiomyocyte survival *in vitro* (Marsano, 2010; Maidhof, 2010; Cerino, 2016). Mechanical stimulation (collaboration with the Politecnico of Milano, Italy) was employed to greatly promote human induced pluripotent stem cell-derived cardiomyocyte maturation and contractility (Marsano, 2016).

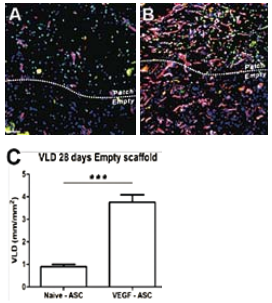

Fig. 1: Representative immunofluorescence images of border between the empty collagen scaffolds and the patches generated by naïve (A) or VEGF-expressing ASC (B) after 29 days *in vivo* (Ve-Cadherin in red; human specific nuclei in green; "staining with" DAPI for cell nuclei in blue). Size bar = 100µm. Graph represents the vessel length density (VLD) assessed in the empty scaffolds after 28 days *in vivo*. Data are represented as mean ± SEM. (n = 3) (B).

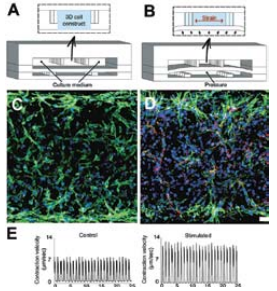

Fig. 2: Design of the 3D heart-on-a-chip microdevice (A-B). Constructs (A) consisted of cardiomyocytes embedded in fibrin gel. By pressurizing the bottom compartment (pressure, B) the PDMS membrane deforms, compressing the 3D cell construct (strain, B). Effects of cyclic strain was evaluated after 5 days in culture by the expression of specific cardiac markers (cardiac Troponin I in green; connexin-43 in red; DAPI used for cell nuclei in blue, C-D) and by the contraction rate during spontaneous beating (control and stimulated micro-tissues E).

Selected Publications

Boccardo S, Gaudiello E, Melly LF, Ricci D, Eckstein F, Martin I, Ban A, Marsano A. (2016) Engineered mesenchymal cell-based patches as controlled VEGF delivery systems to induce extrinsic angiogenesis. Acta Biomaterialia in press

Marsano A, Medeiros da Cunha CM, Ghanaati S, Gueven S, Centola M, Tsaryk R, Barbeck M, Barbero A, Helmrich U, Schaeren S, *et al.* (2016) Spontaneous *in vivo* chondrogenesis of bone marrow-derived mesenchymal progenitor cells by blocking VEGF signaling. Stem Cells Trans Med in Press

Marsano A*, Conficconi C, Lemme M, Occhetta P, Gaudiello E, Votta E, Cerino G, Redaelli A, Rasponi M.* (2016) Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues. Lab Chip. 16(3):599–610.
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Cerino G, Gaudiello E, Grussenmeyer T, Melly L, Massai D, Ban A, Martin I, Eckstein F, Grapow M, Marsano A. (2016) Three dimensional multi-cellular muscle-like tissue engineering in perfusion-based bioreactors. Biotechnol Bioeng. 113(1):226–36

Jalili-Firoozinezhad S, Rajabi-Zeleti S, Mohammadi P, Gaudiello E, Bonakdar S, Solati-Hashjin M, Marsano A, Aghdami N, Scherberich A, Baharvand H, *et al.* (2015) Facile fabrication of egg white macroporous sponges for tissue regeneration. Adv Healthc Mater. 4(15):2281–90

Connection to Clinical Practice

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Engineered tissues with high angiogenic potential to treat chronic cardiac ischemia

Chronic myocardial ischemia causes progressive deterioration of cardiac function and may lead to end-stage heart failure. However, if blood flow is restored, the tissue at the border zone is capable of resuming full function. Surgical revascularization strategies are currently used to re-establish the macro-circulation. However, some patients could also benefit from an adjuvant pro-angiogenic/repair therapy, which aims at promoting the growth of microcirculation and at rescuing the damaged cardiomyocytes. The ultimate goal of the collaboration with the Cardiac Surgery is to investigate a cell-based therapy capable to induce safe, efficacious angiogenesis in a chronic ischemic myocardium. The strategy here pursued is based on the tissue engineering paradigm which provides control over the targeted area -reducing not desired systemic effects- and superior implanted cell survival compared to cell intramyocardial injection delivery. The current research program of the group includes the engineering of 3D patches with high angiogenic potential made by human adipose tissue-derived stromal vascular fraction cells, known to (1) contain subpopulations of both mesenchymal and endothelial progenitor cells and (2) release a broad range of pro-cell-survival factors. Specific induction of microvascular networks and release of cardioprotective factors in the hypo-perfused myocardial areas might be crucial to preserve cardiomyocyte survival and rescue their contraction capability in order to improve the overall cardiac function.