Cardiac Surgery and Engineering

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

The main goal of our group is to investigate repair therapies based on engineered tissues (patches) to induce safe and efficacious angiogenesis and to rescue hibernating myocardium in a chronic ischemic myocardium. The three-dimensional (3D) bioreactor culture of heterogenous Stromal Vascular Fraction (SVF) cells aims to standardize the production of engineered patches (Fig.1). SVF as a cell population is capable of releasing a broad secretome range comprising angiogenic and pro-survival factors. Further aims are to develop *in vitro* functional cardiac models to investigate processes of myocardial repair and regeneration.
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Patches engineered by cells with a high angiogenic and repair potential
In this approach, we use human adipose tissue-derived SVF cells as a heterogeneous cell population with a high angiogenic potential thanks to the presence of numerous endothelial and mural progenitors (collaboration with A. Scherberich, Tissue Engineering Group, Department of Biomedicine, Unibas). We demonstrated that perfusion-based bioreactor culture supported the maintenance of endothelial and mural cells as compared to static culture, thereby accelerating whole construct vascularization and support of cell survival upon implantation in a subcutaneous nude rat model (Fig. 1–2). Moreover, medium conditioned during 3D perfusion- based culture of SVF was showed to partially rescue damaged cardiomyocyte function during monolayer culture under sever hypoxic condition (<1% of oxygen) (Mytsyk and Isu, 2018). An ongoing *in vivo* diseased study aims to investigate the repair potential of SVF-perfused patches in nude rat model of cardiac ischemia (in collaboration with the Department of Cardiac Surgery, USB).

3D functional cardiac models
Our angiogenic engineered tissues might also affect cardiac repair and regeneration by influencing cardiomyocyte maturation and functionality while increasing progenitor cell recruitment. Therefore, we aim to generate a 3D functional cardiac models as a tool to investigate the interactions of SVF cells' secretome and cardiomyocytes. We hypothesize 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, *et al.* 2010; Maidhof, *et al.* 2010; Cerino, *et al.* 2016). Mechanical stimulation (collaboration with the Politecnico of Milano, Italy and Politecnico of Torino, Italy) was employed to greatly promote rat neonatal cardiomyocytes or human induced pluripotent stem cell-derived cardiomyocyte maturation and contractility both at the micro- and macro- scale (Marsano, *et al.* 2016, Massai and Pisani, *et al.* 2020). Microfluidic culture systems harnessing mechanical stimulation was recently generated to recapitulate some of key steps of a scar formation (Occhetta, Isu, *et al.* 2019).
A novel 24-multi-well bioreactor (Patent number: EP19165964) was also developed to culture mm-scale engineered cardiac constructs in a highly controlled environment under multi-physical stimulations (isotonic and auxotonic loads combined with electrical stimulation).

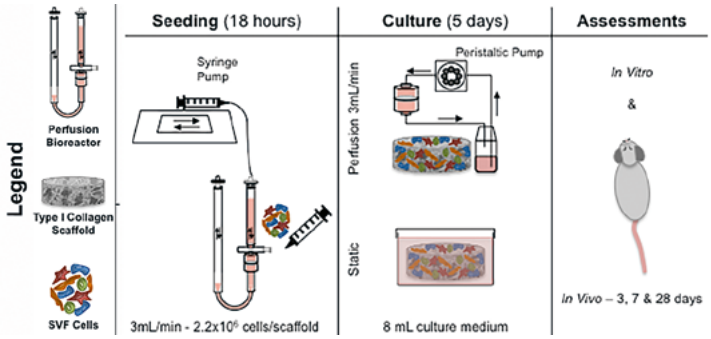

Fig. 1: Scheme of the SVF-based patch study. Summary of the main steps to generate SVF cell-based engineered tissues (patches). SVF cells were seeded on a collagen-based scaffolds under direct alternated perfusion for 18 hours followed by 5 days of culture either under direct uni-directional perfusion or in static condition. The resulted patches were assessed *in vitro* and *in vivo* (in subcutaneous pockets of nude rats).

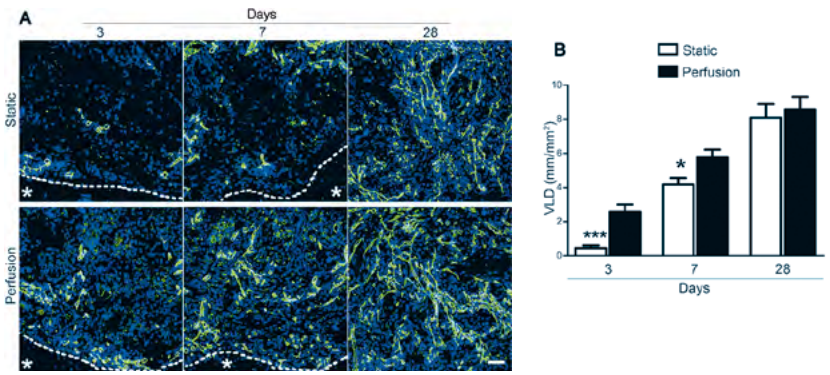

Fig. 2: Perfusion accelerated *in vivo* vascularization of engineered tissues. Engineered patches generated either under perfusion or in static culture were analysed after 3, 7, and 28 days *in vivo*. **(A)** Representative immunofluorescence images stained for endothelial cells (CD31; green). Dashed lines outline the border between the patch and rat tissue (identified by the *). Scale bar = 50 µm. **(B)** VLD quantification normalized over the analysed area.

Selected Publications

Massai D†, Pisani G†, Isu G, Rodriguez Ruiz A, Cerino G, Galluzzi R, Pisanu A, Tonoli A, Bignardi C, Audenino AL, Marsano A† and Morbiducci U† (2020). Bioreactor Platform for Biomimetic Culture and in situ Monitoring of the Mechanical Response of *in vitro* Engineered Models of Cardiac Tissue. Front. Bioeng. Biotechnol. † equally contributing authors.

Isu G, Robles Diaz D, Grussenmeyer T, Gaudiello E, Eckstein F, Brink M, Marsano A. (2020). Fatty acid-based monolayer culture to promote *in vitro* neonatal rat cardiomyocyte maturation. Biochim Biophys Acta Mol Cell Res. 1867(3):118561.

Occhetta P, Isu G, Lemme M, Conficconi C, Oertle P, Räz C, Visone R, Cerino G, Plodinec M, Rasponi M, Marsano A (2018). A three-dimensional *in vitro* dynamic microtissue model of cardiac scar formation. Integr Biol (Camb) 1;10(3):174–183.

Cerino G, Gaudiello E, Muraro MG, Eckstein F, Martin I, Scherberich A, Marsano A (2017). Engineering of an angiogenic niche by perfusion culture of adipose-derived stromal vascular fraction cells. Sci Rep. 27;7(1):14252.

Staubli SM, Cerino G, Gonzalez De Torre I, Alonso M, Oertli D, Eckstein F, Glatz K, Rodríguez Cabello JC, Marsano A (2017). Control of angiogenesis and host response by modulating the cell adhesion properties of an Elastin-Like Recombinamer-based hydrogel. Biomaterials 135:30–41.

Connection to Clinical Practice

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***In vivo* animal models to tackle vascularization issues at the macro- and micro-scale**

Cardiovascular diseases and chronic myocardial ischemia cause progressive deterioration of cardiac function and lead to end-stage heart failure. However, the restore of blood flow both at the macro and micro-level and the additional treatment with cardioprotective factors in the hypo-perfused myocardial areas could preserve cardiomyocyte survival and rescue their contraction, therefore improving the overall cardiac function.

Macro-circulation. Revascularization strategies aim to use autologous blood vessels (e. g. saphenous vein) as coronary artery bypass grafts (CABG). However, some patients might need an alternative to the autologous vessels due to recurrent operations or morbidity issues. Nowadays there still lacks a valid alternative to autologous grafts used for CABG. Our research group has developed a vascular graft made of acetobacteria-derived nano cellulose reinforced by a cobalt-chrome mesh. Previous results showed the cellulose vascular graft's patency and its colonization by host vascular cells following a one-month implantation.

The clinical research counterpart has established two long term studies to test the patency of the cellulose vascular grafts used as carotid artery replacement in sheep and as CABG in pigs. Micro-circulation. 3D SVF cell-based patches as an adjuvant angiogenic and repair therapy could provide control over the targeted area, reducing undesired systemic effects, while enhancing implanted cell survival, compared to intramyocardial cellular injections. A nude rat model of chronic cardiac ischemia was recently established to test the angiogenic and repair potential of SVF cell-based patches.