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Prospective Technologies for Cardiac Repair

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Abstract

Cardiac diseases represent the major cause of death worldwide. Pharmacological treatments, although very sophisticated, are not able to definitively cure cardiac diseases. Furthermore, heart transplantation has shown to be efficient, but unsustainable because of donor shortage and extremely high costs of surgery and patient follow up. Finally, cell therapy applied to the injured myocardium has demonstrated to be inadequate to integrate a sufficient number of efficient contractile cells into the cardiac architecture. Considering the further expansion of cardiac diseases related to the explosive extension of longevity, it is urgent to formulate safe and cost-effective novel strategies to treat cardiac patients, without increasing the economic and social burden on public and private insurances as well as on families. Among others, the "selective repair" of the damaged region of a organ appears as the most reliable approach in the near future. Indeed, recent evidences have suggested that adult progenitor cells can be used to fabricate *ex vivo* engineered cardiac tissue to be implanted into the injured myocardium. However, novel materials and procedures to fabricate bio-compatible scaffolds are necessary to cope with the peculiar heart microenvironment and functional characteristics. In principle,

engineered tissues can be fabricated using biocompatible polymeric scaffolds that remain embedded in the engineered tissue or, alternatively, the scaffold can be stuck on the petri bottom and the new tissue fabricated on, and not around, it. In the latter case, the engineered tissue will be scaffoldless. The current limitation of both technologies is that the scaffold is intended as a mere cell support. Instead, the scaffold must be active part in the array of biological signals governing the formation of a new tissue. This issue is very crucial in the specific case of engineered cardiac tissues that must repeat the native architecture and function. Indeed, preliminary results have shown that specifically manipulated biomaterials can be used to fabricate scaffolds inherently able to deliver signals sensed as "biologically relevant" by cells. The manipulation of the scaffold topology and nanostructure or the use of appropriate composite materials can allow to differentiate stem cells towards the cardiac phenotype in an architectural context very similar to the native one. This new class of scaffolds are very potent in addressing the cell phenotype when fine tuned in respect to the culture medium.

Alternatively, human progenitor cells, possibly isolated from the heart of the same patient candidate to receive the cell treatment, can be used to fabricate scaffoldless tissue sheets. When leant on the heart surface used as a scaffold, the scaffoldless tissue sheets release the embedded progenitor cells that easily migrate into the myocardium differentiating in cardiomyocytes and integrating in the tissue architecture, as demonstrated by the proper connections established between the graft and host cells.

Keywords: Cardiovascular disease, Tissue engineering, Progenitor cells, Additive manufacturing, Bioprinting.

3.1 Medicine Changing Needs

Medicine is undergoing an epochal revolution determined by the expanding ageing and, thus, sickening population, as never before in mankind history. The amplified awareness about the aging-related diseases (myocardial infarction, stroke, diabetes, cancer, etc.) and the advancements in biomedical research together with the increased wealth of industrialized and developing countries have generated great expectation about the possibility of developing very sophisticated treatments for their definitive cure while creating an equal access to the most advanced diagnostic and therapeutic procedures by all individuals independently of the geographic and economic conditions. This vision, besides its ethical and humanitarian relevance, involves very huge economical issues;

in fact, public and private insurances can hardly sustain present and future burden of degenerative diseases.

In this context, particular attention must be paid to cardiovascular diseases (CVD) that represent a growing health and socio-economic burden in most countries around the world [1, 2]. According to WHO, cardiovascular diseases are the most important cause of death and disability worldwide causing 24 million deceases/year by the end of 2030. WHO estimates that low- and middle-income countries are disproportionately affected: over 80% of global CVD deaths occur there proving that CVD is no longer a "rich white man's disease". According to the American Heart Association and the National Heart, Lung and Blood Institute, the staggering costs of treatments for CVD in the USA, including healthcare expenditures and lost productivity due to deaths and disability, were more than 500 billion USD in 2010. In the EU, CVD cause over 1.9 million deaths [3] and determine a total estimated annual cost of 169 billion EUR, with healthcare accounting for 62% of costs, which accounts for 10% of the healthcare expenditure across the Union. Informal care of patients and productivity losses exceeded another 115 billion [4]. However, the burden of CVD should not be measured by deaths alone; the cost in terms of human suffering and lost lives is incalculable. In this scenario, the available treatment options prolong the life span of cardiac patients, but these treatment modalities are not able to provide permanent solution for CVD. Furthermore, heart transplantation as well as implantable artificial heart pumps are available for few patients, only.

The lesson to be drawn from these alarming facts and figures is that the prevention and cure of cardiovascular diseases is not only an important medical necessity, but is also a social and economic imperative for the world sustainable growth and development [5]. Unfortunately, the development of novel efficient drugs requires impressive investments and no patents for major cardiovascular drugs have been registered in the last two decades. Also heart transplantation is restricted to few patients because of very high costs, organ shortage and possible immune rejection. Indeed, an insufficient number of heart transplantations is performed in North America and Europe only, while developing countries cannot afford the costs and, thus, their patients are excluded from this treatment option [6]. Renovated hopes have been raised by the evidence that stem cell therapy displays the potential to regenerate and repair the heart after injury [7, 8]. However, in spite of extensive investments and intensive investigations [9, 10], clinical trials have shown that only marginal benefits on heart function is induced by stem cell therapy in cardiac patients [11]. In fact, protocols and technologies so far used in cardiac cell therapy are rather rudimentary

and do not consider the complexity of the myocardial tissue [12] that is a heterogeneous, anysotropic, viscoelastic system made of inert materials and a multiplicity of cell types (Figures 3.1 and 3.2). Cell therapy based on stem cells suspended in a culture medium and injected into the myocardium display major drawbacks, such as: (a) lack of a consensus on which cells types should be used; (b) lack of control of the potency of these cells; (c) poor cell survival and engraftment; (d) uncertain prevention of uncontrolled differentiation; (e) need for mechanistic understanding of cellular function in the therapeutic setting; (f) inexistence of instructive biomaterials that can promote and induce cell survival, migration and remodeling of the cardiac matrix; (g) lack of strategies to control the environment of the injured tissue to make it more suitable for the graft homing.

Recent advancements in cell biology and biomaterials research as well as the evolution of concepts and vision have indicated that Tissue Engineering could benefit the progress of regenerative medicine and its clinical application. Tissue Engineering is an emerging interdisciplinary field that aims at fabricating on the bench portions of biological tissues that can be used not only

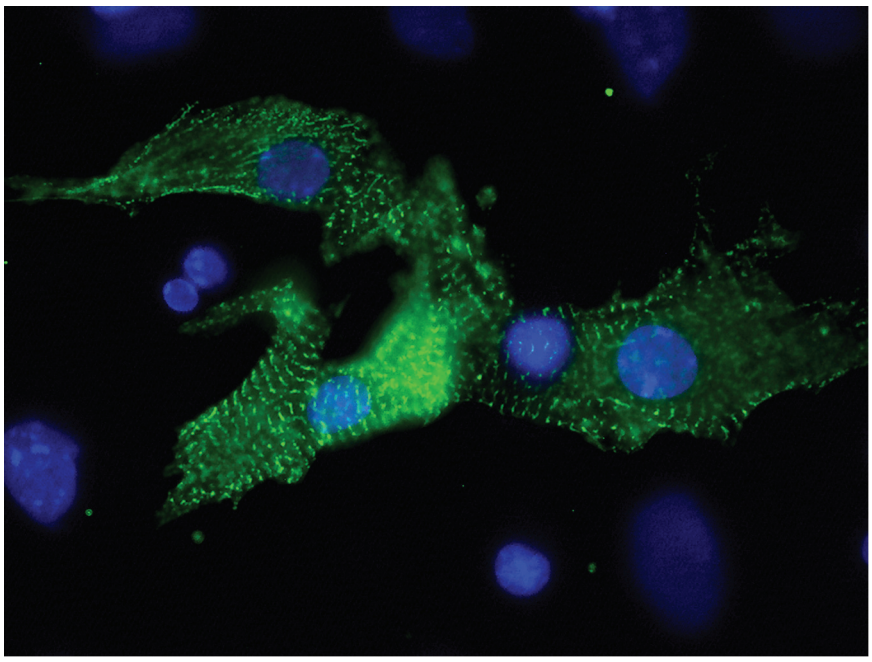

Figure 3.1 Mouse neonatal cardiomyocytes stained with α-sarcomeric actinin in green and nuclei in blue. Magnification 60×.

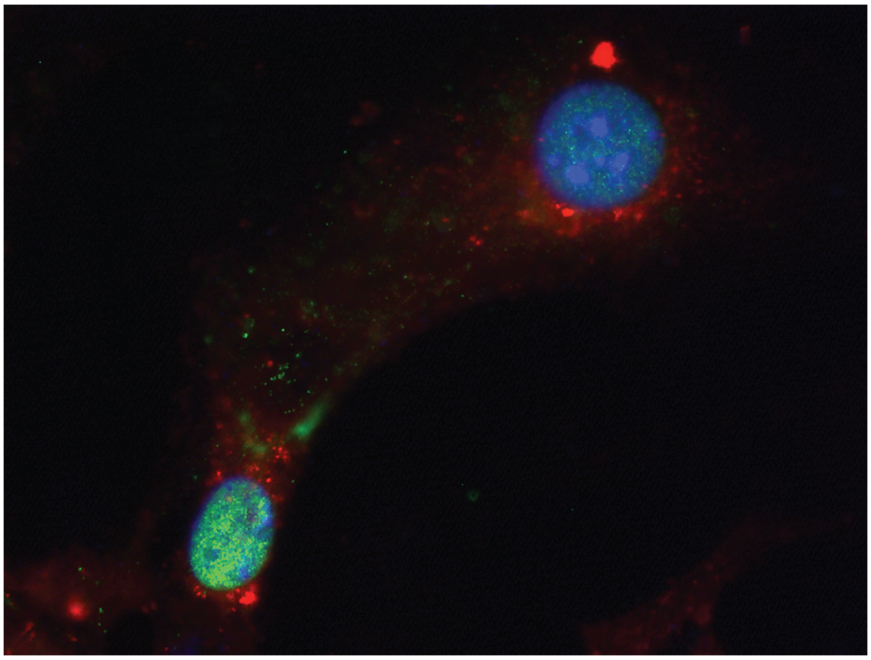

Figure 3.2 Cardiomyogenic differentiation of Vybrant red labeled-human cardiac progenitor cells (hCPCs) co-cultured with mouse neonatal cardiomyocytes. After 1 week, hCPCs show an up-regulation of GATA-4 (green) inside the nuclei. Magnification 60×.

to repair organs suffering from degenerative diseases [13], but also as controllable 3D models to study cell development and drug effects. The availability of human biological tissues will dramatically reduce the demand for whole heart (as well as other organs) transplantations, since only the damaged portions will be repaired overcoming organ shortage.

3.2 Additive Technologies in Tissue Engineering

Manufacturing engineered tissues implies the use of three components: functional scaffolds, cells and an appropriate environment. The fabrication of functional scaffolds is a very complex endeavor that entails the integration of the knowledge so far accumulated in different international laboratories in a multidisciplinary effort to finalize a very intricate procedure of manufacturing and implantation. This also implies that novel expertise must be created for the sake of patients and industries.

To fabricate engineered myocardial tissues, the first and most important achievement for the near future is to design and fabricate a scaffold very closely mimicking the ECM [14]. Innumerable scaffolds for myocardial tissue have been so far designed and experimentally tested, but none of them has demonstrated to be technologically ready for the clinical setting. The next generation of scaffolds must definitively allow cell growth and tissue organization thanks to a controlled architecture characterized by variations of the internal porosity with consequent enhanced control of interconnected channel networks to favor nutrient delivery, waste removal, exclusion of materials or cells, protein transport, and cell migration [13]. In addition, the scaffold must control cell fate releasing biochemical signals delivered by biomolecules (IGF, EGF, IL-1, IL-10, HGF, etc.). So far, biomolecules have been added in the scaffold starting solution with a resulting homogenous distribution into the scaffold itself. These results are quite far from the natural distribution of bioactive molecules that are distributed on the basis of very finely organized gradients [15]. Finally, scaffolds must release physical signals by itself (stiffness, tessellation, topology, etc.) and by incorporated micro/nanosystems able to generate adequate physical stimuli (e.g., electric field).

Different cell types grown on a multitude of scaffolds made of different materials have been so far investigated in order to fabricate strips of myocardial tissue [16–18]. Nevertheless, cell seeded scaffolds encounter host immune response, mechanical mismatch with the surrounding tissue, difficulty in uniformly integrate a high number of cells and limitations in incorporating multiple cell types with positional specificity [19]. Scaffoldless cell sheets [17] have also been manufactured (Figure 3.3), but protocols appear not yet reliable to allow clinical applications. Besides biological and biomaterial issues, manufacturing biological tissues requires sophisticated, rapid, extremely accurate and scalable techniques that are not manually operable. In this respect, a quantum leap has been made when it has been realized that additive manufacturing (3D printing) matches these requirements [20]. 3D printing allows to very precisely fabricate scaffolds layer-by-layer. A particular extension of additive technologies focused on living materials (bioprinting) permits to combine (i) different cell types, (ii) polymeric gels to mimic the extracellular matrix, (iii) immuno-suppressive soluble factors to prevent rejection and (iv) biochemical substances to control the behavior [21]. The ultimate goal is to reproduce complex heterogeneous immuno-privileged/biocompatible biological tissues, either by positioning different cell types in desired locations or by inducing progenitor cells to differentiate

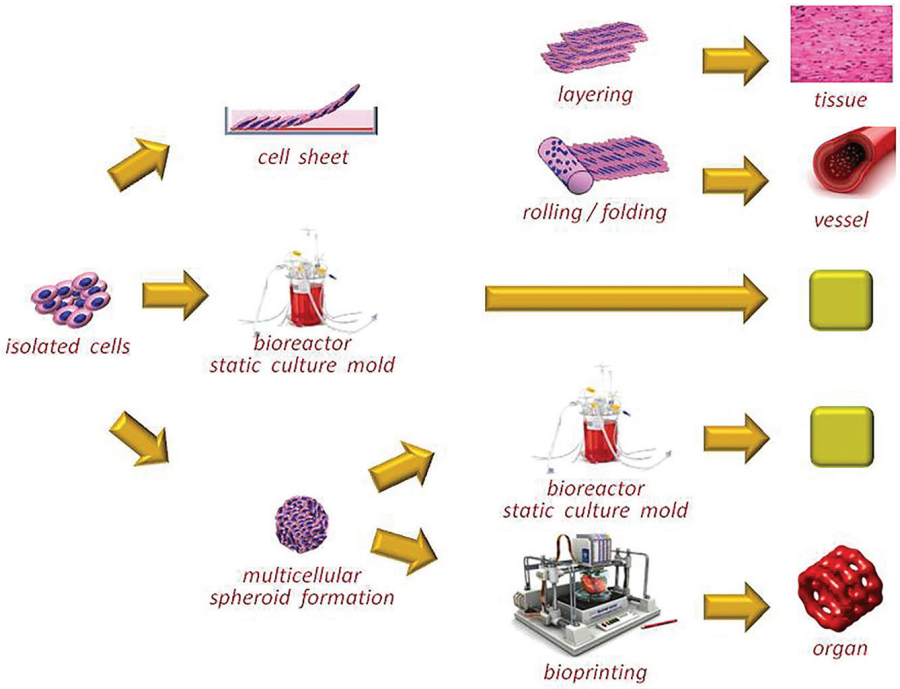

Figure 3.3 Scaffoldless Biofabrication. Engineered tissues can be fabricated in the absence of polymeric structures (scaffolds) supporting cell growth. The figure summarizes the different strategies under experimentation. Techniques to manufacture cell sheets (*upper figure*) are the most simple and generate engineered tissues made of a single cell population. Instead, 3D Bioprinting can allow to closely mimic complex tissue architectures.

into the desired cell types in the context of a specific bio-architecture. In this respect, environmental parameters (such as pH, pressure and geometry of the surrounding space) greatly affect the behavior and the differentiation of stem cells, slowing down or speeding up its dynamics and/or addressing toward different types of differentiation.

The complexity of the tissue to be fabricated cannot be reproduced by manual procedures, as in conventional laboratory techniques. It requires that novel materials, technologies and protocols are exploited or invented through a long-term process actuated by merging the quantum of knowledge resident in different disciplines and international laboratories. Ambitious ideas must be exploited in joint continued collaborative efforts in which the risk and the possible failure are central factor of innovation. In this context, materials and procedures must be strictly standardized involving the knowledge accumulated in a multiplicity of fields, such as biology,

medicine, mathematics, ICT, material science and engineering. An example of this approach is the Additive Manufacturing in which a CAD software governs the nozzles of a 3D printer modified to deposit layer-by-layer and to pattern the biocompatible polymeric gels (biopaper) on which small amounts of cells (bioink) are positioned on the basis of a specific architecture. In this process, the biopaper plays a pivotal role. In fact, stem cells are prone to adopt the final phenotype only when cultured in strictly controlled conditions characterized by a critical array of chemical, biochemical and physical factors, emulating the ECM environment of the original tissue. When adequately manipulated and designed, the biopaper (also without embedded biological molecules) can release signals perceived as biologically relevant by cells, as otherwise demonstrated in studies on cardiomyocyte differentiation [22, 23]. Alternative bioprinting protocols allow fabricating scaffoldless bio-tissues. Cells stick and move together in clumps with liquid-like properties during embryogenesis [24, 25]. A bio-mimetic approach to the fabrication of engineered tissues inspired by the mechanisms presiding over cell self-assembling in the absence of scaffolds during embryogenesis is actively investigated. Genetic and physical interplay drives cells self-assembling in microspheroids that constitute the tissue building blocks [26]. Micro-spheroids are fluidic-like and their fusion is driven by surface tension forces and by the "differential adhesion hypothesis" which postulates that cells of diverse types adhere to each other with different strength due to either quantitative or qualitative differences in cell surface adhesion molecules [27]. A mixed population of differentially adhesive cells evolves in a compartmentalized system in which the less adhesive surround the self-aggregated most adhesive cells. This process is also participated by the cellular tensile forces generated by acto-myosin-dependent cell cortex tension. In a subsequent step, the progressive accumulation of self-produced extracellular matrix restricts cell motility and enhances tissue cohesion modulating tissue fusion processes [28]. The synergistic interaction of self-assembling spheroids and self-assembling matrix material ultimately leads to hierarchically ordered structures inducing the evolution of the cell system from an initial to a more stable state [29–31]. However, in the absence of a solid scaffold, engineered self-assembled tissues must undergo a rapid fluid-solid transition to preserve their shape, composition and integrity.

In a typical bioprinting approach, mechanical extruders place multi-cellular aggregates of definite composition (bioink particles) according to a computer-generated template together with hydrogel (biopaper) constituting

the supporting environment. The post-printing fusion of bioink particles generates organoids taking advantage by early developmental mechanisms such as cell sorting and fusion. Taken together, printing-based tissue engineering technology allows (i) producing fully biological (scaffold-free) small diameter tissues; (ii) it is based on natural shape-forming (i.e. morphogenetic) processes, that are present during normal development; (iii) it can provide organoids of complex topology (i.e, branching tubes); (iv) it is scalable and compatible with methods of rapid prototyping. The ultimate goal of this technology is to generate protocols to instruct (rather than to use) cells to fabricate tissues through a highly engineered artificial environmental milieu. Indeed, the full biological potential of stem cells (*in vitro* or *in vivo*) can be deployed only in an environment mimicking native development. Bioprinting technology holds promise to allow reproducing this complex environment even if an intense investigation activity must be undertaken to fine-tune all the innumerable details that can guarantee a successful output.

References

[1] C.J. Murray, A.D. Lopez. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. Lancet 349(9063):1436–42, 1997.

[2] R. Kahn, R.M. Robertson, R. Smith, D. Eddy, 'The impact of prevention on reducing the burden of cardiovascular disease' Circulation, 118, 576–585, 2008.

[3] S. Petersen, V. Peto, M. Rayner, J. Leal, R. Luengo-Fernandez, A. Gray. European cardiovascular disease statistics, British Heart Foundation.

[4] J. Leal, R. Luengo-Fernández, A. Gray, S. Petersen, M. Rayner. Economic burden of cardiovascular diseases in the enlarged European Union. Eur Heart J. 27: 1610–1619, 2006.

[5] W.S. Weintraub, S.R. Daniels, L.E. Burke, B.A. Franklin, D.C. Jr Goff, L.L. Hayman, D. Lloyd-Jones, D.K. Pandey, E.J. Sanchez, A.P. Schram, L.P. Whitsel; American Heart Association Advocacy Coordinating Committee; Council on Cardiovascular Disease in the Young; Council on the Kidney in Cardiovascular Disease; Council on Epidemiology and Prevention; Council on Cardiovascular Nursing; Council on Arteriosclerosis; Thrombosis and Vascular Biology; Council on Clinical Cardiology, Stroke Council 'Value of primordial and primary prevention for cardiovascular disease: a policy statement from the American Heart Association' Circulation, 124, 967–990, 2011.

[6] C. Benden, L.B. Edwards, A.Y. Kucheryavaya, J.D. Christie, A.I. Dipchand, F. Dobbels, R. Kirk, A.O. Rahmel, J. Stehlik, M.I. Hertz MI, International Society of Heart and Lung Transplantation, 'The Registry of the International Society for Heart and Lung Transplantation: fifteenth pediatric lung and heart-lung transplantation report—2012' J. Heart Lung Transplant., 31, 1087–1095, 2012.

[7] K. Urbanek, D. Torella, F. Sheikh, A. De Angelis, D. Nurzynska, F. Silvestri, C.A. Beltrami, R. Bussani, A.P. Beltrami, F. Quaini, R. Bolli, A. Leri, J. Kajstura, P. Anversa, 'Myocardial Regeneration by Activation of Multipotent Cardiac Stem Cells in Ischemic Heart Failure' Proc. Natl. Acad. Sci. USA., 102, 8692–8697, 2005.

[8] O. Bergmann, R.D. Bhardwaj, S. Bernard, S. Zdunek, F. Barnabé-Heider, S. Walsh, J. Zupicich, K. Alkass, B.A. Buchholz, H. Druid, S. Jovinge, J. Frisén, 'Evidence for Cardiomyocyte Renewal in Humans' Science, 324, 98–102, 2009.

[9] R. Bolli, A.R. Chugh, D. D'Amario, J.H. Loughran, M.F. Stoddard, S. Ikram, G.M. Beache, S.G. Wagner, A. Leri, T. Hosoda, F. Sanada, J.B. Elmore, P. Goichberg, D. Cappetta, N.K. Solankhi, I. Fahsah, D.G. Rokosh, M.S. Slaughter, J. Kajstura, P. Anversa, 'Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial' Lancet, 378, 1847–1857, 2011.

[10] K. Malliaras, R.R. Makkar, R.R. Smith, K. Cheng, E. Wu, R.O. Bonow, L. Marbán, A. Mendizabal, E. Cingolani, P.V. Johnston, G. Gerstenblith, K.H. Schuleri, A.C. Lardo, E. Marbán, 'Intracoronary cardiosphere-derived cells after myocardial infarction: evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction)' J. Am. Coll. Cardiol., 63, 110–122, 2014.

[11] A. Abbott, 'Doubts over heart stem-cell therapy' Nature, 509, 15–16, 2014, Erratum in: Nature, 509, 272, 2014.

[12] P. Di Nardo, G. Forte, A. Ahluwalia, M. Minieri. Cardiac progenitor cells: potency and control. J Cell Physiol 224: 590–600, 2010.

[13] R. Langer, J.P. Vacanti, 'Tissue engineering' Science, 260, 920–926, 1993.

[14] T. Dvir, B.P. Timko, D.S. Kohane, R. Langer, 'Nanotechnological strategies for engineering complex tissues' Nat. Nanotechnol., 6, 13–22, 2011.

[15] E.M. Pera, H. Acosta, N. Gouignard, M. Climent, I. Arregi, 'Active signals, gradient formation and regional specificity in neural induction' Exp. Cell. Res., 321, 25–31, 2014.

[16] G. Forte, S. Pietronave, G. Nardone, A. Zamperone, E. Magnani, S. Pagliari, F. Pagliari, C. Giacinti, C. Nicoletti, A. Musaró, M. Rinaldi, M. Ribezzo, C. Comoglio, E. Traversa, T. Okano, M. Minieri, M. Prat, P. Di Nardo. Human cardiac progenitor cell grafts as unrestricted source of supernumerary cardiac cells in healthy murine hearts. Stem Cells. 29: 2051–2061, 2011.

[17] M. Kawamura, S. Miyagawa, K. Miki, A. Saito, S. Fukushima, T. Higuchi, T. Kawamura, T. Kuratani, T. Daimon, T. Shimizu, T. Okano, Y. Sawa, 'Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model' Circulation, 126, S29–37, 2012.

[18] Y.S. Choi, K. Matsuda, G.J. Dusting, W.A. Morrison, R.J. Dilley, 'Engineering cardiac tissue in vivo from human adipose-derived stem cells' Biomaterials, 31, 2236–2242, 2010.

[19] G. Vunjak-Novakovic, N. Tandon, A. Godier, R. Maidhof, A. Marsano, T.P. Martens, M. Radisic, 'Challenges in cardiac tissue engineering' Tissue Eng. Part B Rev., 16, 169–187, 2010.

[20] B. Derby. Printing and prototyping of tissues and scaffolds. Science 338: 921–926, 2012.

[21] S.V. Murphy, A. Atala, '3D bioprinting of tissues and organs' Nat. Biotechnol., 32, 773–785, 2014.

[22] S. Pagliari, A.C. Vilela-Silva, G. Forte, F. Pagliari, C. Mandoli, G. Vozzi, S. Pietronave, M. Prat, S. Licoccia, A. Ahluwalia, E. Traversa, M. Minieri, P. Di Nardo, 'Cooperation of biological and mechanical signals in cardiac progenitor cell differentiation' Adv. Mater., 23, 514–518, 2011.

[23] F. Pagliari, C. Mandoli, G. Forte, E. Magnani, S. Pagliari, G. Nardone, S. Licoccia, M. Minieri, P. Di Nardo, E. Traversa. Cerium oxide nanoparticles protect cardiac progenitor cells from oxidative stress. *ACS Nano* 6: 3767–3775, 2012.

[24] R.A. Foty, C.M. Pfleger, G. Forgacs, M.S. Steinberg. Surface tensions of embryonic tissues predict their mutual envelopment behavior. *Development* 122: 1611–1620, 1996.

[25] G. Forgacs, R.A. Foty, Y. Shafrir, M.S. Steinberg, 'Viscoelastic properties of living embryonic tissues: a quantitative study' Biophys J., 74, 2227–2234, 1998.

[26] V. Mironov, R.P. Visconti, V. Kasyanov, G. Forgacs, C.J. Drake, R.R. Markwald. Organ printing: tissue spheroids as building blocks. Biomaterials 30: 2164–2174, 2009.

[27] R.A. Foty, M.S. Steinberg. The differential adhesion hypothesis: a direct evaluation. Dev. Biol., 278, 255–263, 2005.

[28] M. McCune, A. Shafiee, G. Forgacs, I. Kosztin. Predictive modeling of post bioprinting structure formation. Soft Matter 10: 1790–800, 2014.

[29] E. D. F. Ker et al. Bioprinting of growth factors onto aligned sub-micron fibrous scaffolds for simultaneous control of cell differentiation and alignment. *Biomaterials* 32: 8097–107, 2011.

[30] K. Jakab, C. Norotte, B. Damon, F. Marga, A. Neagu, C.L. Besch-Williford, A. Kachurin, K.H. Church, H. Park, V. Mironov, R. Markwald, G. Vunjak-Novakovic, G. Forgacs. Tissue engineering by self-assembly of cells printed into topologically defined structures. *Tissue Eng Part A* 14: 413–421, 2008.

[31] J.A. Phillippi, E. Miller, L. Weiss, J. Huard, A. Waggoner, P. Campbell. Microenvironments engineered by inkjet bioprinting spatially direct adult stem cells toward muscle- and bone-like subpopulations. *Stem Cells* 26: 127–134, 2008.