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Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration

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ABSTRACT

Recent study showed that mesenchymal stem cells (MSC) could inhibit apoptosis of endothelial cells in hypoxic condition, increase their survival, and stimulate the angiogenesis process. In this project we applied Laser-Induced-Forward-Transfer (LIFT) cell printing technique and prepared a cardiac patch seeded with human umbilical vein endothelial cells (HUVEC) and human MSC (hMSC) in a defined pattern for cardiac regeneration. We seeded HUVEC and hMSC in a defined pattern on a Polyester urethane urea (PEUU) cardiac patch. On control patches an equal amount of cells was randomly seeded without LIFT. Patches were cultivated *in vitro* or transplanted *in vivo* to the infarcted zone of rat hearts after LAD-ligation. Cardiac performance was measured by left ventricular catheterization 8 weeks post infarction. Thereafter hearts were perfused with fluorescein tomato lectin for the assessment of functional blood vessels and stored for histology analyses. We demonstrated that LIFT-derived cell seeding pattern definitely modified growth characteristics of co-cultured HUVEC and hMSC leading to increased vessel formation and found significant functional improvement of infarcted hearts following transplantation of a LIFT-tissue engineered cardiac patch. Further, we could show enhanced capillary density and integration of human cells into the functionally connected vessels of murine vascular system. LIFT-based Tissue Engineering of cardiac patches for the treatment of myocardial infarction might improve wound healing and functional preservation.

1. Introduction

A cardiac patch can provide a support matrix which allows stem/progenitor cell adhesion and proliferation in a damaged heart [1–3]. It is a possible alternative to the current approach of direct cell injection for cell-based therapy. However, inadequate neovascularization remains the major limitation in clinical application of cardiac patch. Due to the impaired nutrients and oxygenation perfusion after myocardial infarction (MI), cardiac tissue formation will be confined to limited area with only marginal functional improvement.

Laser-Induced-Forward-Transfer (LIFT) derived from industrial electronic manufacturing demonstrates advantages for controlled transfer of inorganic and biological materials in conjunction with proteins, peptides, DNA, RNA and cells [4]. A precise arrangement of cells could be obtained in three-dimensional (3D) patterns [5]. Refinement of this methodical approach achieved survival rates of printed cells of nearly 100%. There were no signs of DNA-damage, increased apoptosis rates or any effect on the proliferation [4]. The advantage of LIFT is the potential to form a precise multilayered 3D construct in a single step. The technique allows integration of an endothelial cell pattern for vascular network formation to construct a myocardium analog with precise 3D organization.

Mesenchymal stem cells or multipotent stromal cells (MSC) are capable of self-renewing and have the potential to differentiate into multiple lineage [6]. MSC were tested in animal models for a number of diseases [7,8] and in clinical trials for myocardial ischemia with promising angiogenic outcome [9]. MSC have

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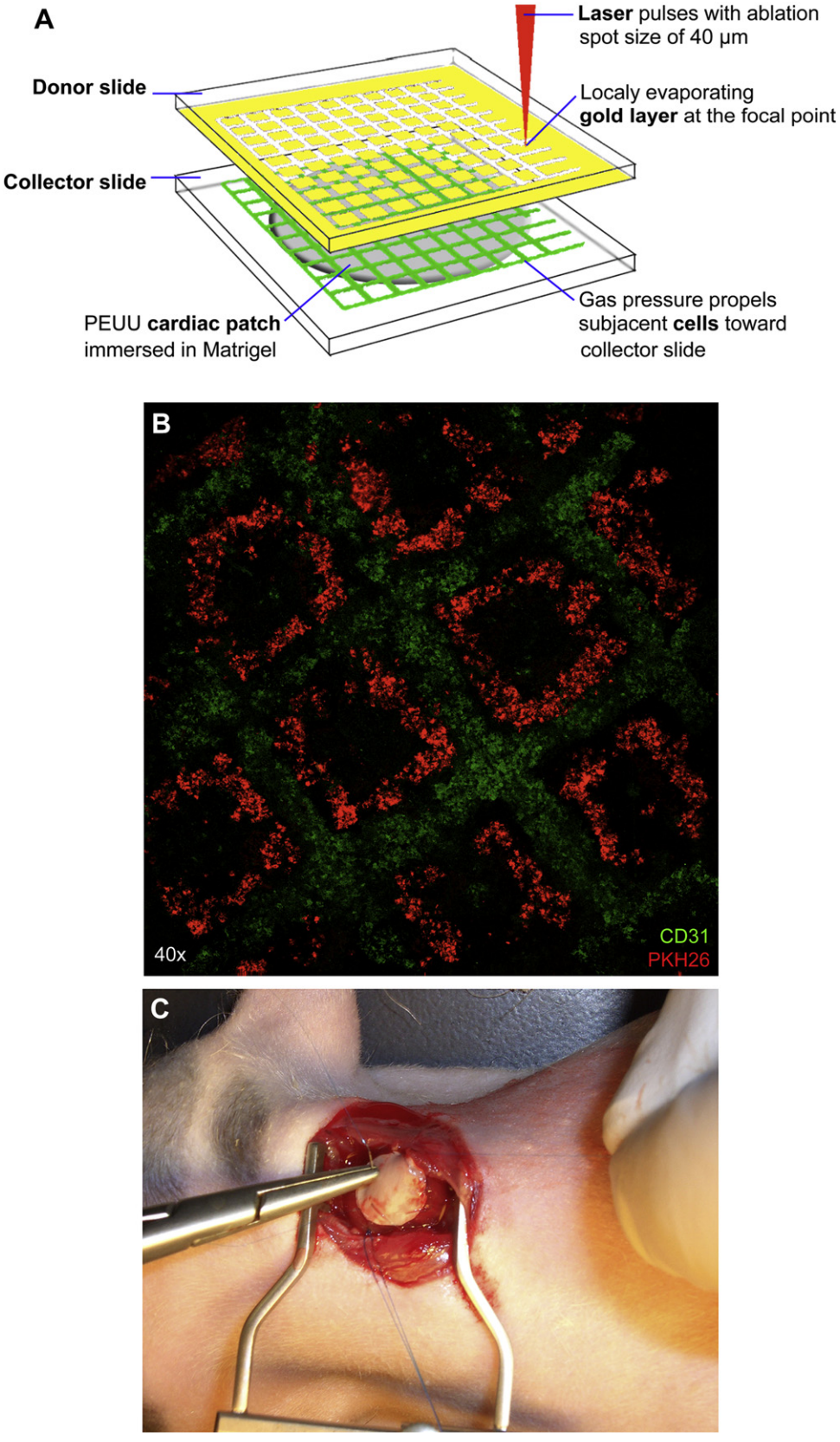

Fig. 1. Experimental design: A. Schematical bioprinting setup based on LIFT. B. Arrangement of transferred cells by LIFT observed after 24 h: Human MSC were prestained with PKH26 (SIGMA-Aldrich, St. Louis, USA) and patches were stained with polyclonal goat anti-Pecam1 (Santa Cruz Biotechnology) 24 h after LIFT to separate grid patterned HUVEC. C. Patch implantation *in vivo*: After LAD-ligation rats received the cardiac patch sutured onto the area of blanched myocardium.