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CELL IMPLANTATION

New technique for needle-less implantation of eukaryotic cells

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Abstract

Background aims. On review of the use of stem cells in the literature, promissory outcomes for functional organ recovery in many subspecialties in medicine underscore its therapeutic potential. The application of stem cells through the use of a needle can result in additional scar formation, which is undesired for delicate organs. The present work describes the use of a needle-less stem cell injector with the Immediate Drop on Demand Technology (I-DOT) for cell injection *in vitro*. *Methods.* Mesenchymal stromal cells from human bone marrow were labeled with ethynyl-deoxyuridine (EdU) for 2 days and then were re-suspended. With the use of I-DOT, the cells were applied to type 1 collagen matrices or pig bladder tissue specimens with or without mucosa at different levels of energy. The collagen matrices were analyzed after 4 h and 5 days; bladder tissue specimens were analyzed 4 h after cell implantation. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test (MTT) assay was performed immediately after cell application to the collagen matrices. Histological analysis with the use of frozen sections and immunofluorescence was used to localize EdU-labeled cells. *Results.* A considerable number of cells were detected by use of the MTT assay for collagen matrices. In the collagen matrix, the mean measured depth immediately after application ranged between 210 μm and 489 μm, 220 μm and 270 μm for entire bladder specimens, and 230 μm and 370 μm for bladder without mucosa. Cells survived for up to 5 days in the collagen matrix in both bladder specimens. *Conclusions.* Cells can survive during I-DOT application, which suggests that the I-DOT device may be a potentially suitable technology for needle-less cell application onto tissues.

Key Words: *cell injection, cell therapy, collagen matrix, ethynyldeoxiuridine, needle-less, stem cells*

Introduction

In the past decade, much research regarding cell therapy has been published. In particular, the use of stem cells has been considerably investigated and proposed as a treatment option for an enormous range of conditions, including organ regeneration. With a better understanding of pluripotent cell biology, stem cells may potentially be applied to a broad spectrum of therapies once promissory results have shown a systemic or local therapeutic effect on the basic and preclinical research levels [1–3].

Cell injection is the most commonly used method for implantation of living cells into deeper tissues or organs. Stem cells have been injected into a broad range of tissues including the vocal cord [4], anal sphincter [5] and urethral sphincter [6,7]. However, the use of needles for injection of cells has disadvantages. The local tissue injury caused by needle insertion may enhance scar tissue formation and inflammatory reactions, which, in turn, can be harmful for injected cells and may negatively interfere with the desired effect or result in suboptimal outcomes. Although needle-less devices for drug injection or vaccination are described [8,9], these techniques are not suitable for tissue implantation of living eukaryotic cells. Dispensing liquids in droplets on micrometer-scales or nano-scales was reported for applications other than cell injections [10,11]. However, these techniques may offer substantial stress for living cells in the suspension. The use of

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micro-shockwaves is an alternative to the use of needles and can penetrate up to depths of 100 μm [12]; however, it may not suitable for cell therapy because the rapid onset of pressure after the blast may rupture the cells present in different parts of a laminar flow under effect of Poiseuille law [13].

To date, needle-less implantation of living eukaryotic cells has not yet been described in the literature. A new automated air-displacement system, the Immediate Drop on Demand Technology (I-DOT), developed and patented by the Fraunhofer IPA, may be a promising alternative solution for implantation of cells [14]. With the use of the I-DOT, a short, compressed air pulse is applied to the fluid-containing the cells in a source well with the use of a nozzle at the bottom to release a defined amount of liquid. Droplets with a defined volume in the nano-liter range can be spotted onto tissue or matrices.

Methods

Isolation and culture of mesenchymal stromal cells

This study was approved by the Karl Eberhard University of Tübingen ethics committee. Bone marrow (BM) aspirates, taken from the iliac crest during radical prostatectomy or cystoprostatectomy, were provided by the Department of Urology, University of Tübingen, after written consent from the patient. Human mesenchymal stromal cells (MSCs) were isolated from the BM on the basis of previous protocols [15,16]. In brief, approximately 10 mL of BM was collected and mixed with 5000 IU of heparin (Sigma-Aldrich). Mononuclear cells were recovered with the use of Ficoll Histopac (Ficoll-paque plus; Amersham Biosciences; 400*g*, 30 min, room temperature). Mononucleated cells in the interphase were collected, washed with phosphate-buffered saline and seeded in 75-cm^2 cell culture flasks (BD Bioscience) at a density of 2×10^5 cells/cm^2. After 24 h of incubation in fresh-frozen plasma platelet (FFPP) medium at 37°C, 5% CO_2 in a humidified atmosphere, non-adherent cells were removed. MSCs were expanded in animal serum–free FFPP medium as described [17]. MSCs at the second passage were used for the experiments. The characterization of the MSCs was confirmed through immunohistochemistry (data not shown).

MSC labeling

MSCs were irreversibly labeled with the use of ethynyl-deoxiuridine (EdU). Briefly, after cell expansion the cells were cultured for 2 days in 5 μmol/L EdU in Dulbecco's Modified Eagle Medium in T-75 flasks until cells were approximately 80% confluent. Cells were then trypsinized and consecutively implanted with the use of the I-DOT technology.

A sample of labeled MSCs at the time of I-DOT implantation were cultured overnight and then were fixed in ice-cold acetone for 5 min and stained with EdUClick (Invitrogen) in the dark, according to the manufacturer's recommendation. For analysis of the percentage of labeled cells, the slides were incubated with Hoechst 33342 (1:2000) for 30 min in the dark.

Collagen-based matrices

Insoluble type I collagen fibers (Symathese) were used to prepare molecularly defined collagen matrices as previously described [18]. In brief, A 0.5 wt % type I collagen suspension in 0.25 mol/L acetic acid was prepared and incubated for 16 h at 4°C, homogenized (4°C), de-aerated by centrifugation at 250*g* for 15 min at 4°C and put into a 24-well plate, aiming to construct 3-mm-thick collagen matrices. The collagen solution was then subjected to 4-h freezing (−20°C) followed by lyophilization. The matrices were not cross-linked.

Bladder acquisition

The bladder dome was harvested from mini-pigs that were killed during other experiments not involving the urinary tract at the animal laboratory facility at the Karl Eberhard University of Tübingen. This study was approved by the Animal Ethics Committee. Euthanasia was performed through the use of a barbituric (pentobarbital) overdose. The specimens were transported in 18% saccharose. The bladder dome was cut into eight 1-cm^2 pieces. In four pieces, the overlining mucosa was mechanically removed through dissection with scissors after saline injection in the submucosal layer for tissue detachment. The I-DOT cell implantations were performed 6 h after tissue harvesting.

I-DOT cell implantation

The cultivated and labeled MSCs were trypsinized and re-suspended at a final concentration of 5×10^6 MSC/mL ion Dulbecco's Modified Eagle Medium. The I-DOT source well, with a nozzle of ∅60 μm, was filled with approximately 60 μL of the MSC solution (5×10^6/mL). The source well was applied directly and at close contact over the collagen matrices or bladder specimens. At least four spots of cells were applied per specimen, as shown in Figure 1, at defined energy levels.

Two energy levels were tested for cell implantation: E1 and E2. The low energy (E1) level was applied with the use of a valve opening time of 0.01