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Effect of 2-octylcyanoacrylate on placenta derived mesenchymal stromal cells on extracellular matrix

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ABSTRACT

Purpose: Determine the effect of 2-octylcyanoacrylate on placenta derived mesenchymal stromal cells (PMSCs) seeded onto extracellular matrix (ECM) in order to assess its biocompatibility as a potential adhesive for *in-vivo* fetal cell delivery.
Methods: PMSCs isolated from chorionic villus tissue were seeded onto ECM. A MTS proliferation assay assessed cellular metabolic activity at various time points in PMSC-ECM with direct, indirect, and no glue contact. Conditioned media collected prior to and 24 hours after glue exposure was analyzed for secretion of human brain-derived neurotrophic factor, hepatocyte growth factor, and vascular endothelial growth factor.
Results: Direct and indirect contact with 2-octylcyanoacrylate results in progressively decreased cellular metabolic activity over 24 hours compared to no glue controls. Cells with direct contact are less metabolically active than cells with indirect contact. 24 hours of glue exposure resulted in suppression of growth factor secretion that is near complete with direct contact.
Discussion: Exposure to 2-octylcyanoacrylate results in decreased metabolic activity and decreased measurable secretion of growth factors by PMSCs seeded onto ECM. Thus, the application of 2-octylcyanoacrylate glue should be limited when working with cell-engineered scaffolds as its inhibitory effects on cell growth and secretory function can limit the therapeutic potential of cell-based interventions.

1. Introduction

The growth of the field of fetal surgery has created opportunities to incorporate *in utero* interventions with mesenchymal stromal cell (MSC) based therapy [1,2]. There is evidence that MSCs may play a role in wound healing, immune modulation and cytoprotection [3–6] and thus incorporating MSCs with existing surgical therapies may improve clinical outcomes. Placenta derived mesenchymal stromal cells (PMSCs) are an optimal source of MSCs given their unique origin in the fetal environment and potential for autologous therapy. Studies have shown that PMSCs are capable of differentiating into cells expressing bone, muscle, neural and cardiac markers and can likely further augment emerging fetal surgical interventions [1,3,7,8]. Previous work by our laboratory has demonstrated that PMSCs are compatible with a variety of delivery matrices which can help facilitate their therapeutic effect upon surgical transplantation [1]. Thus, the construction of an optimal delivery vehicle for cell-based interventions has created a novel field of tissue-engineered scaffolds. Extracellular matrix (ECM) derived from Small Intestinal Submucosa is well studied as a potential delivery vehicle for MSC based therapies and possibly has positive synergistic effects when used in conjunction with MSCs [9–11]. The logistics of translating PMSCs seeded onto ECM for use in the clinical setting is a field ripe for investigation.

2-octylcyanoacrylate glue (Dermabond, Ethicon) is a commonly available medical grade adhesive that was first introduced in 1998 [12]. The majority of its clinical application is limited to superficial wound closures and control of vascular hemorrhage [13] however animal studies have shown additional efficacy in vascular repair [14], middle ear surgery [15], and mesh fixation in hernia repair [16,17]. The literature also reports its use in conjunction with matrix

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material for drug delivery systems [18,19] and dural closures [20–22]. Animal models of the fetal surgical repair of myelomeningocele have reported use of cyanoacrylate (CA) glues to help secure bioengineered patches to the defect [23–25] however it remains unclear the impact the glue has on the cell population. CA based glues are synthesized by condensation of cyanoacetate with formaldehyde in the presence of a catalyst [26]. Despite its widespread application, the glue has been reported to have various cytotoxic properties. Activation of cyanoacrylate based glue releases heat and can cause cellular damage [27]. During polymerization, formaldehyde is also released which may cause cellular toxicity [28]. Few studies have also demonstrated *in vitro* cytotoxic effects of CA glues in cell culture [26–28].

Thus, the purpose of our study is to determine the cytotoxicity of 2-octylcyanoacrylate on placenta derived mesenchymal stromal cells seeded onto an acellular small intestinal submucosa extracellular matrix patch to assess its biocompatibility as an adhesive for *in-vivo* cell delivery.

2. Material and methods

2.1. Isolation and culture of PMSCs from human second trimester placenta

Three discarded de-identified samples of placenta tissue (15–19 weeks gestational age) were collected at the University of California Davis Medical Center. PMSCs were isolated using an explant culture method previously reported by our laboratory and cryopreserved in liquid nitrogen after isolation [1,2,29]. The three cell lines were manufactured and fully characterized as MSCs as previously described [30]. Briefly, cells at passage 3 underwent multipotency analysis and flow cytometry to detect the presence of MSC cell surface markers. Cryopreserved cells were thawed at passage 5 and seeded onto ECM at passage 6 for all experiments. All experiments were performed in triplicate. The culture medium used for all experiments was Dulbecco's Modified Eagle Medium (DMEM) high glucose with 5% fetal bovine serum (FBS), 100 U/ml Penicillin (Invitrogen), 100 μg/ml streptomycin (Invitrogen), 20 ng/ml basic fibroblast growth factor (R&D Systems), and 20 ng/ml recombinant human epidermal growth factor (R&D Systems).

2.2. Green fluorescent protein transduction of PMSCs, cell seeding and imaging

Cells were transduced with a green fluorescent protein (GFP)-containing lentiviral vector (UC Davis Institute for Regenerative Cures Vector Core Facility), seeded onto 6 mm diameter circular four-ply ECM discs (Small Intestine Submucosa (SIS), Cook Biotech Inc., West Lafayette, Indiana) at a density of 3×10^5 cells/cm^2 [9], and cultured in a 24-well plate. Cells seeded onto ECM were imaged using a Carl Zeiss Axio Observer Z1 Microscope. ECM was inverted prior to imaging to allow visualization of the cells.

2.3. Glue exposure

The direct glue exposure setting was created by applying a drop of 2-octylcyanoacrylate to the center of the cell seeded ECM. In the indirect glue exposure group, a drop of 2-octylcyanoacrylate was applied to the periphery of the well containing the cell seeded ECM taking care to avoid any contact with the cell seeded ECM. Control groups included: cell seeded ECMs without exposure to glue, ECM only without glue, and glue only.

2.4. Cell proliferation assay

Cellular metabolic activity for each group at pre glue exposure, 2, 6 and 24 hours (h) post glue exposure was determined using a 3-(4,5-dimethylthizazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium (MTS) based colorimetric assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega). The assay was performed according to the manufacturer's instructions. Absorbance at 490 nm wavelength was quantified using a Molecular Devices SpectraMax i3 plate reader instrument (Molecular Devices LLC., Union City, California). Values were normalized to the background absorbances of the ECM and glue controls.

2.5. Enzyme linked immunosorbent assays

Culture supernatant collected prior to glue exposure and 24 h after glue exposure was used to quantify secretion of growth factors using enzyme-linked immunosorbent assays (ELISAs). Human brain-derived neurotrophic factor (BDNF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) levels were quantified using Duoset ELISA kits from R&D Systems. All ELISAs were performed according to the manufacturer's instructions and absorbance was measured using a Molecular Devices SpectraMax i3 plate reader instrument (Molecular Devices LLC., Union City, California).

2.6. Statistical analysis

Statistical analysis was performed using a One-way or Two-way ANOVA with repeated measures followed by Tukey's multiple comparisons test in PRISM 7 (GraphPad Software, Inc). Mean values and standard error of the means are reported unless otherwise noted.

3. Results

3.1. Morphological analysis

PMSCs seeded onto ECM demonstrate normal spindled cell morphology and adherence to the matrix (Fig. 1A). After 24 h of direct glue exposure, cells could not be visualized on the ECM (Fig. 1B). After 24 h of indirect glue exposure, cells appeared scant in number and those that remained attached to the ECM were rounded and demonstrated loss of its normal spindle architecture (Fig. 1C) compared to cell seeded ECM without glue controls (Fig. 1D).

3.2. Metabolic activity

Following incubation with the glue for 2, 6 and 24 h, metabolic activity of the cells was quantified using the MTS proliferation assay (Fig. 2). At 2 h, there was a statistically significant difference between the no glue control group and the direct glue group ($p \leq 0.0001$) and the indirect glue group ($p \leq 0.001$). This significant difference persisted at 6 and 24 h ($p \leq 0.01$ for both direct and indirect). There was no statistically significant difference between the direct and indirect glue groups at any time point. Cellular metabolic activity decreased with both direct and indirect exposure to 2-octylcyanoacrylate at all time points compared to control cells. The effect was evident at 2 h and confirmed at 24 h. Metabolic activity of the cells changed significantly with time ($p < 0.0001$) and depending on exposure to glue ($p = 0.0083$). The rate of change over time was also significantly different between the three groups (direct contact, indirect contact, no glue contact) ($p = 0.0026$).

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