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Effects of Polymer Encapsulated Glial Cell Line-Derived Neurotrophic Factor Secreting Cells on Odontoblast-like Cell Survival

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Abstract

Objective: Cells that are encapsulated into immunoprotective and implantable devices provide a means of enabling targeted, long-term, continuous, de novo synthesized delivery of very high levels of therapeutic molecules. In this study, we examined effects of polymer encapsulated glial cell line-derived neurotrophic factor (GDNF) secreting ARPE-19 cells on the in vitro viability of odontoblast-like cells (KN-3 cells) under normally lethal starvation conditions.

Materials and methods: Devices (polymeric hollow fibers) were loaded with either GDNF-secreting ARPE-19 cells (GDNF-device) or non-modified ARPE-19 cells (ARPE-control device). KN-3 cell survival and function was quantified under starvation conditions when the cells were co-cultured with either control or GDNF devices. We further examined the specificity of GDNF by neutralizing the activity of GDNF with the soluble GDNF receptor (GFRα-1).

Results: When KN-3 cells were co-cultured with either device under starvation, the cells exhibited a concentration gradient-dependent enhancement of survival in the area immediately surrounding the device. Similar survival benefits occurred when KN-3 cells were cultured in media previously used to maintain the cells encapsulated in the device. Because ARPE-19 cells also normally secrete low levels of VEGF, the maintenance medium from each device group was pretreated with soluble Flt-1 or soluble GFRα-1 to neutralize VEGF or GDNF. The survival of KN-3 cells was suppressed when cultured in VEGF-neutralized medium from ARPE-control devices, but not in that from GDNF-devices. In contrast, when GDNF was neutralized with soluble GFRα-1, the survival of KN-3 cells was intensely suppressed.

Conclusion: These results suggest that GDNF delivered from encapsulated cells can augment the survival of odontoblast-like cells, and that the delivery of growth factors by encapsulated cells may be useful for enhancing pulp wound healing and regeneration. These data also pave the way for additional in vivo studies to determine the utility of this approach in regenerative endodontics.

Keywords

GDNF; VEGF; Encapsulated cells; Odontoblast; Dental pulp; Starvation; Cell survival

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Introduction

Dental pulp plays an essential role in tooth nutrition and detection of potential pathogens. Damage or loss of dental pulp frequently leads to increased fragility of the tooth and a reduction in quality of life. Accordingly, effective strategies are needed to enhance dental pulp wound healing and regeneration following damage or disease. Regenerative endodontics is a relatively new field of research with the goal of replacing damaged/diseased pulp tissues with regenerated pulp-like tissues. In general, the approaches under investigation include the use of stem cells to replace dental pulp cells [1] and the enhancement of regenerative signaling mechanisms often using material-based scaffolds to deliver therapeutic molecules capable of augmenting regrowth of damaged cells [2].

Our group is developing a delivery system based on implanting trophic factor-secreting cells that have been encapsulated in a polymer membrane. The pores of the membrane are sufficiently large to allow secreted molecules to cross the membrane and enter the surrounding host tissue, but small enough to protect the encapsulated cells from host immune identification. Encapsulated cell therapy provides a targeted, continuous, de novo synthesized source of molecules from a system that combines the potency of de novo in situ synthesis of cell-derived molecules with the safety of an implantable, biocompatible, and retrievable medical device.

Glial cell line-derived neurotrophic factor (GDNF) is known as a member of the transforming growth factor-ß superfamily [3] and has well documented roles in cell survival and regeneration, especially within the central nervous system (CNS) [4]. In addition, GDNF has non-neuronal functions in tooth development [5] and in the survival of dental pulp cells [6], raising the possibility that GDNF could be used to improve wound healing and regeneration of dental pulp.

In the present study, we provide the first evidence that the sustained delivery of GDNF from encapsulated, genetically-modified cells can enhance and prolong the survival of odontoblast-like cells under in vitro long-term starvation conditions.

Material and Methods

Cell culture

ARPE-19 cells were cultured in T-175 flasks with growth medium; Dulbecco's Modified Eagle Medium (DMEM) + glutamax (1x) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific Incorporaiton, MA, USA). Routine culture consisted of feeding the cells every 2-3 days and passaging them at 70-75% confluence. Cells were incubated at 37 °C, 90% humidity and 5% CO_2. A rat clonal dental pulp cell line (KN-3 cells) with odontoblastic properties such as a high level of alkaline phosphatase (ALP) activity was previously established from dental pulp cells of rat incisors [7,8]. KN-3 cells were cultured with minimum essential medium Eagle alpha modification (αMEM) (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% heat-inactivated FBS (JRH Bioscience, Lenexa, KS, USA), 100 mg/mL streptomycin (Sigma-Aldrich), and 100 U/mL penicillin (Sigma-Aldrich) at 37°C in an atmosphere of 5% CO_2 and air.

GDNF cell line establishment

Human GDNF cDNA optimized for human cell line expression was produced by Invitrogen (Thermo Fisher Scientific Incorporation, MA, USA) and subsequently cloned to replace NGF in the expression vector pT2.CAn.hNGF, resulting in the plasmid pT2.CAn.hoG. ARPE-19 cells were transfected with this vector using the Sleeping Beauty (SB) transposase system [9,10]. Briefly, cells were co-transfected with plasmids pT2.CAn.hoG and the SB vector pCMV-SB-100x. As the SB vector does not contain a eukaryotic selection marker cassette, it is only transiently expressed. The transient expression window allows for the active, transposase-mediated integration of the SB transposon, i.e. the inverted repeat SB substrate sequences and the sequences contained within these repeats, including the GDNF expression and neomycin antibiotic resistance cassettes. Clones were selected using G418 (Sigma-Aldrich) and single colonies were expanded and isolated based on their GDNF release levels.

Device fabrication

Cells were encapsulated into porous hollow fiber membranes (the typical pore diameter is <1 μm) as previously described [11]. Devices were manufactured from polysulfone membrane internally fitted with filaments of polyethylene terephthalate yarn scaffolding for cell adhesion. Prior to filling, cultured cells were suspended in human endothelial serum free medium (heSFM) (Gibco) at a density of 10,000 cells/μL and injected into each device using a custom manufactured automated cell-loading system. Devices were loaded with either GDNF-producing cells (GDNF-device) or with non-modified ARPE-19 cells (ARPE-control device) and were separately maintained in a well of 24-well plate with 1 mL of heSFM at 37°C in an atmosphere of 5% CO_2 and air. Maintenance medium of the device was changed in every 5 days.

Scanning electron microscopy analysis

Scanning electron microscopy (SEM) was used to evaluate the ultrastructure of the device membrane wall, as well as the inner and the external surfaces of the device membrane. For visualization of the wall, a freeze-fracture technique was employed prior to coating with metal. Samples were immersed in liquid nitrogen for approximately 2 min and fractured using two pairs of jeweler's forceps. Samples were mounted on carbon adhesive backing on SEM stubs and sputter-coated with a Pd-Au target for 3.5 min. The Hitachi 2700 Scanning Electron Microscopy (Hitachi High Technologies America Incorporation, Clarksburg, MA, USA) was used with an accelerating voltage of 8 kV.

Enzyme-linked immunosorbent assay

Concentrations of GDNF and VEGF secreted from devices were analyzed by enzyme-linked immunosorbent assay (ELISA). GDNF- and ARPE-control devices were individually maintained in 1 mL of heSFM or αMEM supplemented with 0.1% FBS for 7 days. The media was collected and the concentrations of GDNF and VEGF in the medium were determined using human GDNF Emax Immuno Assay System (Promega, Madison, WI, USA) and Quantikine ELISA Human VEGF Immunoassay (R&D systems, Minneapolis, MN, USA) according to the manufacturer's protocol, respectively. Absorbance was determined using a microplate reader (Bio-Rad iMark, Bio-Rad Laboratories, Hercules, CA, USA). GDNF and VEGF in the maintenance medium treated with either soluble Flt-1 or soluble GFRα-1 were also analyzed by ELISA.

Morphological analysis of the cells co-cultured with devices

Twenty four hours before assay, GDNF-device and ARPE-control devices were individually placed into wells of 24-well plate with 1 mL of αMEM supplemented with 0.1% FBS. KN-3 cells (3×10^3 cells/well) in 24-well plates were cultured in αMEM with 10% FBS for 24 hours, followed by a change to αMEM with 0.1% FBS, and then each device was gently immersed into an individual well. During the co-culture, the medium was not exchanged. Seven days after the co-culture, the cells proximal and distal to each device were observed using phase-contrast microscopy.

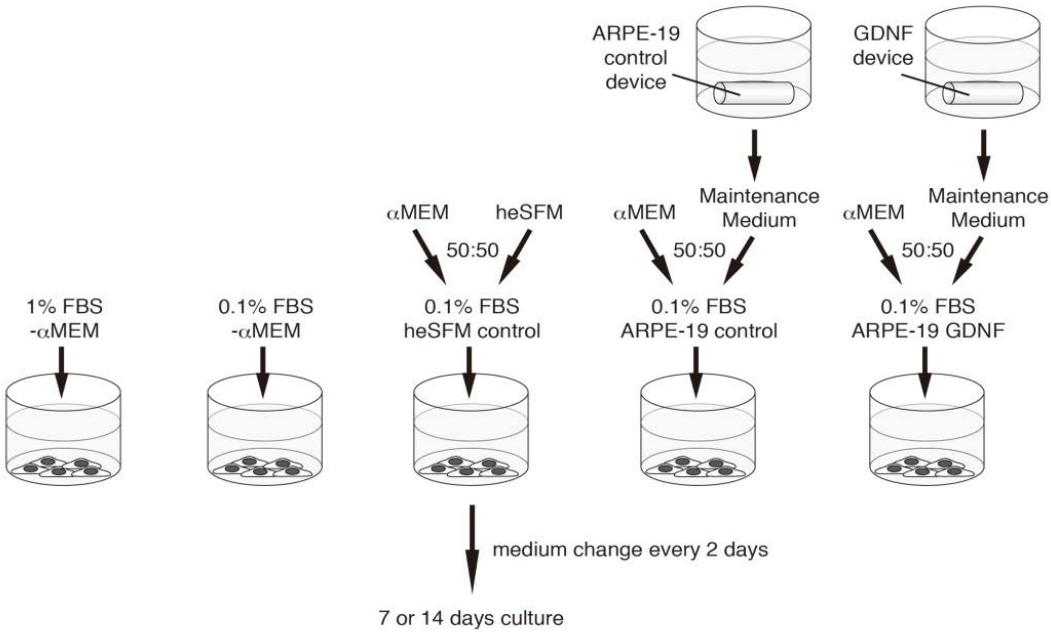

Figure 1: Schema of cell viability assay. Details are explained in Materials and Methods.

Cell viability assay

Cell viability was analyzed by direct counting of viable cells that excluded trypan blue. A diagram of this experiment is shown in Figure 1. The cells (3×10^3 cells/well for 7 days and 1×10^3 cells/well for 14 days) were cultured in 24-well plates containing αMEM with 10% FBS for 24 hours, then the medium was changed to one of the following; 1% FBS-αMEM, 0.1% FBS-αMEM, 0.1% FBS heSFM control; 0.1% FBS mixture of αMEM and heSFM in a ratio of 50:50, 0.1% FBS ARPE-19 control; 0.1% FBS mixture of αMEM and the maintenance medium of ARPE-19-control devices in a ratio of 50:50, and 0.1% FBS ARPE-19 GDNF; 0.1% FBS mixture of αMEM; and the maintenance medium of GDNF-devices in a ratio of 50:50.

The cells were cultured in each medium for 7 or 14 days with the medium change every 2 days. Attached cells were washed with phosphate buffered saline and detached after a brief exposure to trypsin/EDTA. An aliquot (10 mL) of cell suspension was mixed with an equal volume of trypan blue and placed into a TC10 automated cell counter (Bio-Rad Laboratories). Viable cells were counted using trypan blue exclusion. The proliferation of KN-3 cells following the end of long-term starvation was also examined. Each medium was changed to αMEM with 1% FBS at the end of long-term starvation, and cultured for 2 days, followed by counting of viable cells using trypan blue exclusion.

ALP staining and activity

KN-3 cells (3×10^3 cells/well) were cultured in 24-well plates in each conditioned medium for 7 days, then the medium was changed to αMEM with 1% FBS and cultured for additional 1 and 3 days. At each time point, the adherent cells were fixed and stained for ALP using a NBT/BCIP Stock Solution (Sigma-Aldrich) according to the manufacturer's instructions. Also, the cells (4.5×10^3 cells/well) in 6-well plates were cultured in each conditioned medium for 7 days, followed by additional 1 and 3 days in αMEM with 1% FBS, at which time ALP activity was measured using a p-nitrophenylphosphate assay (LabAssay ALP Kit; Wako Pure Chemical Industries, Tokyo, Japan). After 15 min of incubation at 37°C, absorbance of p-nitrophenylphosphate at 405 nm was determined using a microplate reader (Bio-Rad iMark, Bio-Rad Laboratories), and the specific activity of ALP (U/μL) was calculated. One unit of the enzymatic activity is defined as release of 1 nmol p-nitrophenyl per min at pH 9.8 and 37°C.

Neutralization of VEGF by soluble Flt-1 and GDNF by soluble GFRα-1

Before the cell viability assay, 2 mg/mL of soluble Flt-1 (recombinant human VEGF R1/Flt-1 Fc Chimera, 321-FL, R&D systems) was added to each maintenance medium and incubated for 3 hours to neutralize VEGF [12]. The cells were then cultured in each conditioned medium pretreated with soluble Flt-1 for 7 days. Each medium was changed to fresh soluble Flt-1-neutralized medium every 2 days. Seven days after culture, cell viability was analyzed by trypan blue exclusion. Also, the maintenance medium of GDNF-device was pretreated by 3 mg/mL of soluble GFRα-1 (recombinant rat GFRα-1/GDNF Rα-1 Fc Chimera, 560-GR, R&D systems) for 3 hours before the cell viability assay. The cells were cultured in soluble GFRα-1-neutralized medium for 7 days, with the change of the medium to fresh soluble GFRα-1-neutralized medium every 2 days. The cell viability was analyzed by trypan blue exclusion 7 days after the culture.

Statistical analysis

All experiments were performed at least three times to test the reproducibility of results, and the representative results are shown. Results of ELISA, the cell viability assay, and ALP activity are presented as mean ± standard deviation (SD) of three replicates. Statistical significant differences were determined using one-way ANOVA computation combined with Tukey's post hoc tests (IBM SPSS Statistics, Version 19; IBM SPSS, Chicago, IL, USA). Values of $p<0.05$ and <0.01 were regarded as significant.

Results

SEM analysis of a device

Figure 2 shows the general appearance (Figure 2A) and SEM cross sections of an encapsulation device. The membrane of the device possessed a typical isoreticulated morphology with a relatively dense, thin outer skin and an open, much thicker macroporous substructure (Figures 2B, C, D). Measures of membrane cross sections revealed an inner diameter of approximately 480 μm, an outer diameter of 660 μm, and a corresponding wall thickness of approximately 90 μm. In Figure 2E, the function of a device is conceptualized. Genetically-modified GDNF-secreting ARPE-19 cells or non-modified ARPE-19 cells are individually loaded into a device. The cells are retained within the device, and GDNF and/or VEGF produced by encapsulated cells are secreted to the surrounding medium through the pores of the device. Nutrition for encapsulated cells is taken through pores.

Concentrations of GDNF and VEGF secreted from devices

GDNF and VEGF secretion from devices in human endothelial serum free medium (heSFM) was quantified by ELISA. GDNF levels were 5.9 mg/day for GDNF-devices and 0 mg/day for ARPE-control devices. Because ARPE-19 cells normally secrete low levels of VEGF, VEGF was also measured by ELISA and found that VEGF levels were ranged from 0.59-0.67 ng/day, for GDNF- and ARPE-control devices, respectively. When devices were maintained in αMEM with 0.1% FBS, the secretion of both growth factors decreased. In αMEM with 0.1% FBS, concentrations of GDNF and VEGF secreted from GDNF-devices were 0.15 mg/day and 0.4 ng/day, and VEGF secreted from ARPE-control devices was 0.37 ng/day, respectively.

Cell proliferation in co-culture of KN-3 cells with devices

The survival of KN-3 cells was examined after the co-culture with GDNF- or ARPE-control device in αMEM with 0.1% FBS for 7 days (Figure 3). When KN-3 cells were co-cultured with GDNF-device, proliferation of the cells with notable processes was observed in surrounding area of the device (Figure 3B-a), whereas only a few living cells were observed in the far area of the devices (Figure 3B-b). A similar concentration gradient-dependent effect was seen when KN-3 cells were co-cultured with ARPE-control devices. Proliferation of the cells with processes was observed in surrounding area of the device (Figure 3C-a), but not in the far area (Figure 3C-b).

Cell survival after long-term starvation

From the result of ELISA, the secretion of GDNF and VEGF from devices decreased in αMEM with 0.1% FBS than in heSFM, so the cell viability assay was carried out using the medium which the maintenance medium of the device and αMEM were mixed in an equal amount (Figure 1).

Figure 4 shows the survival of KN-3 cells under normal (1%

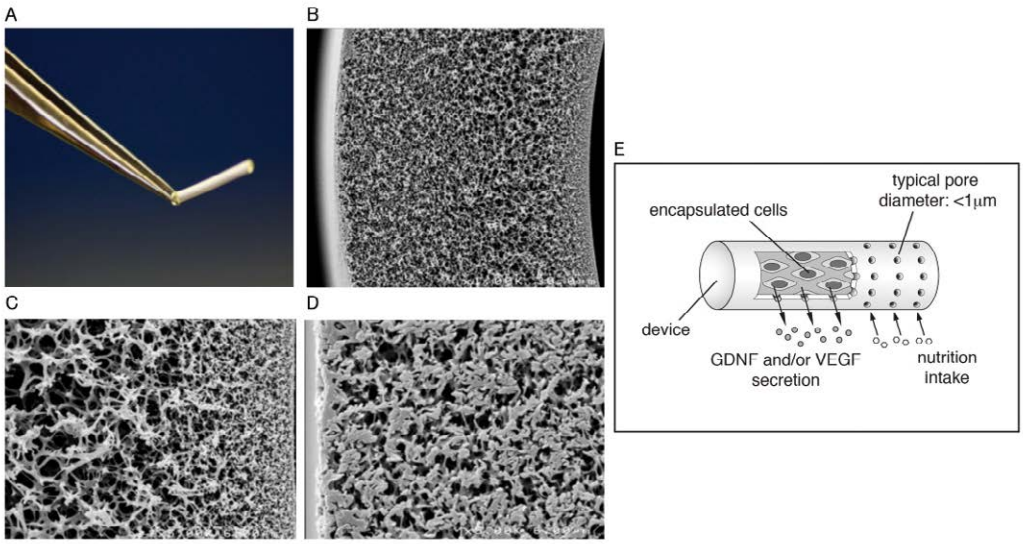

Figure 2: Structure of a device. **A.** A photograph of an intact 7 mm long cell-loaded device. **B, C, and D.** SEM cross-section photographs of the device. Each photograph for device membrane wall (**B**), inner aspect (**C**), and outer aspect (**D**) shows typical isoreticulated pore structure. 5,000x. **E.** Schema of the function of a device. Genetically-modified GDNF-secreting ARPE-19 cells and non-modified ARPE-19 cells are individually encapsulated in a device.

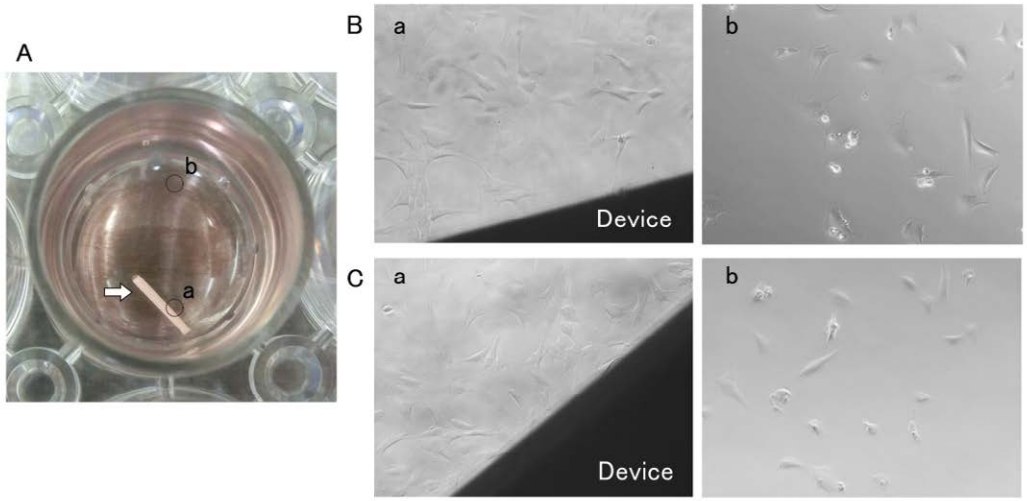

Figure 3: Co-culture of KN-3 cells with devices under starvation. **A.** A photograph of the device (arrow). Representative area around and far from the device are shown as **a** and **b** (dotted-line circle), respectively. **B.** KN-3 cells co-cultured with a GDNF-device. Numerous cells with processes were observed around the device (**a**), whereas only a few cells, some of which were dead, were observed in the area far from the device (**b**). **C.** KN-3 cells co-cultured with a APRE-control device. Again, numerous cells with processes were observed around the device (**a**), but not in the area far from the device (**b**).

FBS-αMEM) and starvation conditions. After 7 days of culture, the number of living cells in 0.1% FBS heSFM control group was about one-third that of the 1% FBS-αMEM group, and the numbers of living cells in both 0.1% FBS ARPE-19 control and 0.1% FBS ARPE-19 GDNF groups were significantly greater than those in both 0.1% FBS-αMEM and 0.1% FBS heSFM control groups (Figure 4A). After 14 days of culture, the number of living cells in 0.1% FBS ARPE-19 control group appeared to be greater than that of 0.1% FBS heSFM control group, and the number of living cells in 0.1% FBS ARPE-19 GDNF group was significantly greater than that of 0.1% FBS heSFM control group (Figure 4B).

Cell proliferation after long-term starvation

To analyze the proliferative ability of surviving KN-3 cells post long-term starvation, the cells after the end of long-term starvation were cultured in αMEM with 1% FBS for 2 days and the numbers of living cells were determined (Figure 5). After 7 day starvation, the numbers of living cells from the 0.1% FBS heSFM control, 0.1% FBS ARPE-19 control, and 0.1% FBS ARPE-19 GDNF groups were significantly greater than that from 0.1% FBS-αMEM group (Figure 5A). After 14 days of starvation, the number of living cells of 0.1% FBS ARPE-19 GDNF group was significantly greater than that from 0.1% FBS heSFM control group, and the number of living cells of 0.1% FBS

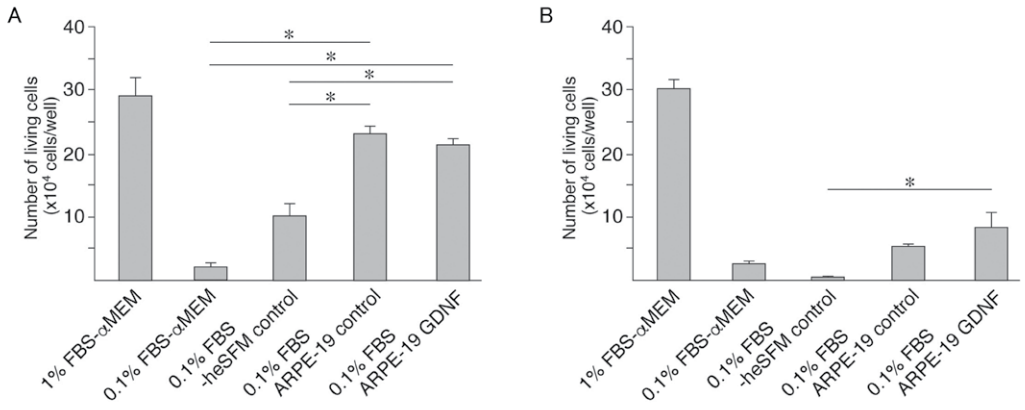

Figure 4: Survival of KN-3 cells under long-term starvation. **A.** Survival of KN-3 cells after 7 days starvation. **B.** Survival of KN-3 cells after 14 days starvation. *p<0.05 (Tukey's test).

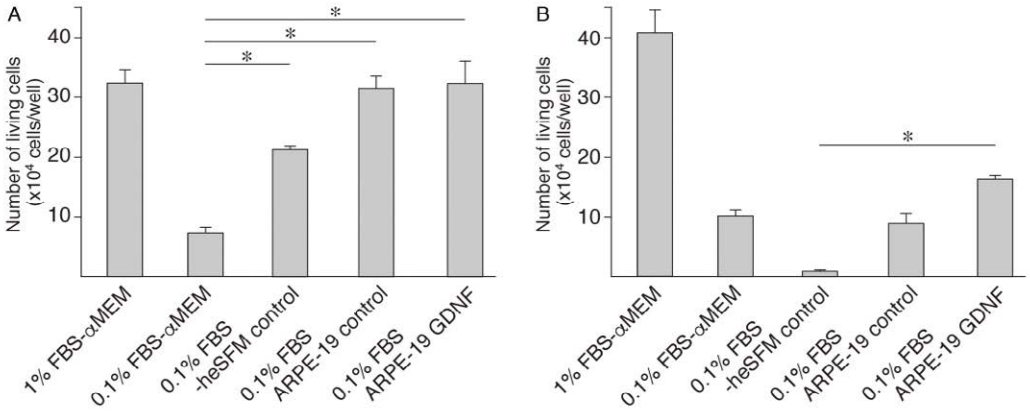

Figure 5: Recovery of the proliferation ability of surviving KN-3 cells after 7 days (**A**) and 14 days (**B**). After the long-term starvation, KN-3 cells were cultured in αMEM with 1% FBS for further 2 days. *p<0.05 (Tukey's test).

ARPE-19 control group was comparable to that of 0.1% FBS-αMEM control (Figure 5B).

Surviving KN-3 cells post 7 day starvation were cultured in αMEM with 1% FBS for an additional 1 and 3 days, and then processed for ALP staining and quantification of ALP activity. ALP staining was observed in the cells of 0.1% FBS-αMEM, 0.1% FBS ARPE-19 control, and 0.1% FBS ARPE-19 GDNF groups, but not in that of 0.1% FBS heSFM control group (Figure 6). ALP activity of 0.1% FBS-αMEM, 0.1% FBS ARPE-19 control, and 0.1% FBS ARPE-19 GDNF groups increased in a time dependent manner (Figure 7).

Effects of neutralization of VEGF and GDNF on the cell survival

Figure 8 shows the effects of either soluble Flt-1 or soluble GFRα-1 on cell survival after long-term starvation. In the 0.1% FBS ARPE-19 control group, neutralization of VEGF with soluble Flt-1 significantly reduced the number of living cells. In contrast, there was no difference in the number of living cells in the 0.1% FBS ARPE-19 GDNF group with or without the neutralization of VEGF by soluble Flt-1. When the maintenance medium of GDNF-device was pretreated by soluble GFRα-1 to neutralize GDNF, the number of living cells in the 0.1% FBS ARPE-19 GDNF group was intensely reduced.

Discussion

Cell encapsulation is a maturing platform technology that has emerged as a viable therapeutic option capable of providing targeted, long-term, continuous, de novo synthesized delivery of very high levels of therapeutic molecules in regenerative therapy [13]. The size of encapsulation devices can be tailored for implantation directly into the desired region of virtually any organ or tissue where the structure of the device retains the enclosed cells, allows the molecules of interest to be secreted, and also prevents immunological reactions of host; all while maintaining the viability of the encapsulated cells [14-16]. In the present study, the in vitro effects of GDNF and VEGF delivered from encapsulation devices were examined, and several basic findings were noted; (a) GDNF and VEGF from the device were capable of imparting significant survival benefits to odontoblast-like cells under long-term starvation conditions, (b) these benefits were concentration gradient-dependent and most pronounced in the region proximal to the device and diminished with increasing distance from the device,

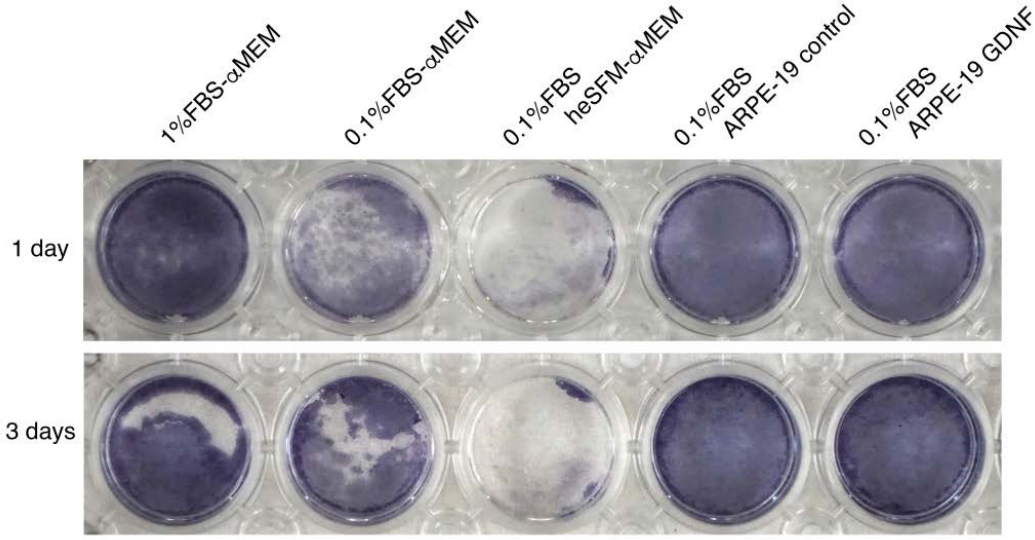

Figure 6: ALP staining of surviving KN-3 cells. After the end of the long-term starvation with each medium, surviving KN-3 cells were cultured in the medium with αMEM with 1% FBS for additional 1 and 3 days, then ALP staining were analyzed.

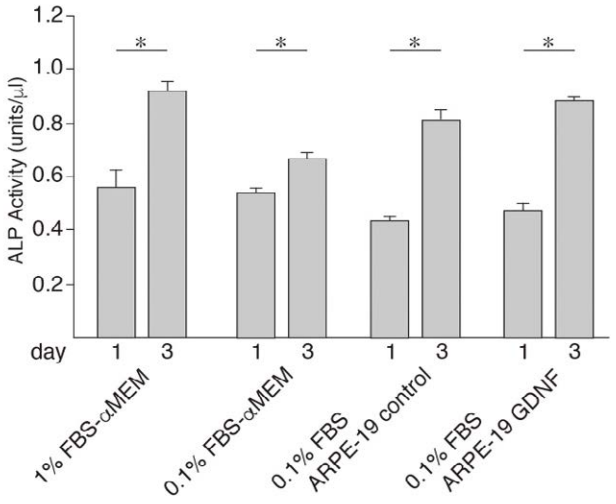

Figure 7: ALP activity of surviving KN-3 cells. After the end of the long-term starvation with each medium, surviving KN-3 cells were cultured in the medium with αMEM with 1% FBS for additional 1 and 3 days, then ALP activity were analyzed. *p<0.05 (Tukey's test).

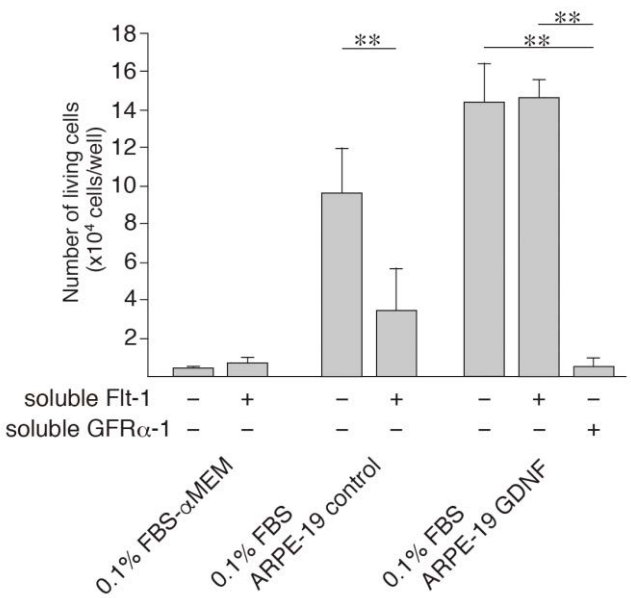

Figure 8: Inhibition of the survival of KN-3 cell by neutralization of VEGF or GDNF. During the starvation, KN-3 cells were cultured in each following medium; 0.1% FBS-αMEM, 0.1% FBS ARPE-19 control, and 0.1% FBS ARPE-19 GDNF with or without the addition of soluble Flt-1 or soluble GFR-α1. **p<0.01 (Tukey's test).

and (c) the surviving cells retained their function as demonstrated by normal proliferative and process elongation capabilities.

Surprisingly, odontoblast-like cells were significantly protected from the consequences of long-term starvation when the maintenance medium of both GDNF- and ARPE-control devices were used for the mixed medium. The maintenance medium of GDNF-device included both GDNF and low levels of VEGF, whereas the maintenance medium of ARPE-control device included only low levels of VEGF. The initial results of the cell viability assay suggested that VEGF contributed to the survival of odontoblast-like cells but the contribution of was unclear since the engineered ARPE-10 cells secreted both GDNF and VEGF. To clarify effects of GDNF secreted from GDNF-device on the cell survival, each maintenance medium was pre-treated with soluble Flt-1 as an inhibitor of VEGF, or soluble GFRα-1 as an inhibitor of GDNF, to neutralize each growth factor in the maintenance medium. As a result, the survival of odontoblast-like cells was suppressed by the addition of soluble Flt-1 to the maintenance medium of ARPE-control device, whereas the cell survival by the maintenance medium of GDNF-device was not inhibited by soluble Flt-1. On the other hand, the cell survival was completely inhibited by the addition of

soluble GFRα-1 to the maintenance medium of GDNF-device. These results indicate that GDNF itself secreted from GDNF-device prolongs the survival of odontoblast-like cells with or without VEGF. In the neutralization assay, soluble Flt-1 did not completely inhibit the survival of odontoblast-like cells. Also after 7 days of starvation, some living cells were observed when cultured in the mixed medium of αMEM and human endothelial serum free medium, suggesting some benefit of the media itself. This effect dissipated after 14 days, further suggesting that the prolongation of the cell survival was induced by GDNF and VEGF secreted from devices, not by human endothelial serum free medium.

Multiple roles of GDNF and VEGF have been demonstrated in a variety of studies. Several research have indicated that the delivery or the induction of GDNF can prolong and protect the survival of photoreceptors of eye [17], spinal cord [18], and bone marrow mesenchymal stem cells [19]. During tooth development, GDNF and its receptors are expressed in dental pulp supporting a role for GDNF in epithelial mesenchymal interactions [20]. In GDNF knockout mice odontoblast differentiation is disrupted and GDNF inhibits dental pulp cell death induced by serum deprivation, suggesting that GDNF may play a cytoprotective role in dental pulp homeostasis during stress conditions and pulpal necrosis [5]. VEGF is known to affect the survival and the proliferation of endothelial cells [21]. In addition to its potent angiogenic abilities, VEGF produced by dental pulp cells can act in an autocrine manner to promote the chemotaxis, proliferation, and/or differentiation of dental pulp cells [22]. Further ongoing experiments are now attempting to clarify the mechanism (s) underlying the effects of GDNF and VEGF secreted from encapsulation devices on the survival of odontoblast-like cells, as well as to clarify effects of them on other cell types such as cementoblasts and periodontal ligament cells.

Conclusions

The in vitro results of the present study indicate that both GDNF and VEGF delivered from encapsulated cells exerted positive survival and proliferative influences on odontoblast-like cells. When GDNF- and/or VEGF-secreting devices are used for dental pulp in the clinic, it should be size-adapted and structurally optimized, however, the present results also suggest the possibility of using this encapsulated cell-based delivery system for multimodal polypharmacy in dentistry.

Acknowledgements

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References

1. Lohara K, Zhenge L, Ito M, Ishhizaka R, Nakamura H, et al. (2009) Regeneration of dental pulp after pulpotomy by transplantation of CD31-/CD146- side population cells from a canine tooth. Regen Med 4: 377-385.
2. Ishimatsu H, Kitamura C, Morotomi T, Tabata Y, Nishihara T, et al. (2009) Formation of dentinal bridge on surface of regenerated dental pulp in dentin defects by controlled release of fibroblast growth factor-2 from gelatin hydrogels. J Endod 35: 858-865.
3. Airaksinen MS, Saarma M (2002) The GDNF family: signalling, biological functions and therapeutic value. Nat Rev Neurosci 3: 383-394.
4. Ibáñez CF, Andressoo JO (2017) Biology of GDNF and its receptors - Relevance for disorders of the central nervous system. Neurobiol Dis 97: 80-89.
5. de Vicente JC, Cabo R, Ciriaco E, Laurà R, Naves FJ, et al. (2002) Impaired dental cytodifferentiation in glial cell-line derived growth factor (GDNF) deficient mice. Ann Anat 184: 85-92.
6. Gale Z, Cooper PR, Scheven BAA (2011) Effects of glial cell line-derived neurotrophic factor on dental pulp cells. J Dent Res 90: 1240-1245.
7. Nomiyama K, Kitamura C, Tsujisawa T, Nagayoshi M, Morotomi T, et al. (2007) Effects of lipopolysaccharide on newly established rat dental pulp-derived cell line with odontoblastic properties. J Endod 33: 1187-1191.
8. Ozeki N, Mogi M, Yamaguchi H, Hiyama T, Kawai R, et al. (2014) Differentiation of human skeletal muscle stem cells into odontoblasts is dependent on induction of α1 integrin expression. J Biol Chem 289: 14380-14391.
9. Fjord-Larsen L, Kusk P, Emerich DF, Thanos Torp CM, Bintz B, et al. (2012) Increased encapsulated cell biodelivery of nerve growth factor in the brain by transposon-mediated gene transfer. Gene Ther 19: 10-17.
10. Fjord-Larsen L, Kusk P, Tornøe J, Juliusson B, Torp M, et al. (2010) Long-term delivery of nerve growth factor by encapsulated cell biodelivery in the Göttingen minipig basal forebrain. Mol Ther 18: 2164-2172.
11. Tornøe J, Torp M, Jørgensen JR, Emerich DF, Thanos Bintz CB, et al. (2012) Encapsulated cell-based delivery of Meteorin is neuroprotective in the quinolinic acid rat model of neurodegenerative disease. Restor Neurol Neurosci 30: 225-236.
12. Yi KW, Jung SH, Cho GJ, Seol HJ, Hong SC, et al. (2014) Effects of sFlt-1 and alpha 2-macroglobulin on vascular endothelial growth factor-induced endothelin-1 upregulation in human microvascular endothelial cells. Placenta 35: 64-69.
13. Emerich DF, Orive G, Thanos C, Tornoe J, Wahlberg LU (2014) Encapsulated cell therapy for neurodegenerative diseases: from promise to product. Adv Drug Deliv Rec 67-68: 131-141.
14. Desai T, Shea LD (2017) Advances in islet encapsulation technologies. Nat Rev Drug Discov 16: 367.
15. Kauper K, McGovern C, Sherman S, Heatherton P, Rapoza R, et al. (2012) Two-year intraocular delivery of ciliary neurotrophic factor by encapsulated cell technology implants in patients with chronic retinal degenerative diseases. Invest Ophthalmol Vis Sci 53: 7484-7491.
16. Lathuilière A, Laversenne V, Astolfo A, Kopetzki E, Jacobsen H, et al. (2016) A subcutaneous cellular implant for passive immunization against amyloid-β reduces brain amyloid and tau pathologies. Brain 140: 1587-1604.
17. Wong FS, Wong CC, Chan BP, Lo AC (2016) Sustained Delivery of Bioactive GDNF from Collagen and Alginate-Based Cell-Encapsulating Gel Promoted Photoreceptor Survival in an Inherited Retinal Degeneration Model. PLoS One 11: e0159342.
18. Freeman KA, Fullerton DA, Foley LS, Bell MT, Cleveland JC Jr, et al. (2015) Spinal cord protection via alpha-2 agonist-mediated increase in glial cell-line-derived neurotrophic factor. J Thorac Cardiovasc Surg 149: 578-586.
19. Gao X, Du J, Fan G, Yang C, Deng L, et al. (2014) Adenoviral-mediated GDNF protects bone marrow mesenchymal stem cells against apoptosis induced by hydrogen peroxide. Biomed Mater Eng 24: 2169-2176.
20. Hellmich HL, Kos L, Cho ES, Mahon KA, Zimmer A (1996) Embryonic expression of glial cell-line derived neurotrophic factor (GDNF) suggests multiple developmental roles in neural differentiation and epithelial-mesenchymal interactions. Mech Dev 54: 95-105.
21. Domigan CK, Warren CM, Antanesian V, Happel K, Ziyad S, et al. (2015) Autocrine VEGF maintains endothelial survival through regulation of metabolism and autophagy. J Cell Sci 128: 2236-2248.
22. Matsushita K, Motani R, Sakuta T, Yamaguchi N, Koga T, et al. (2000) The role of vascular endothelial growth factor in human dental pulp cells: induction of chemotaxis, proliferation, and differentiation and activation of the AP-1-dependent signaling pathway. J Dent Res 79: 1596-1603.

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