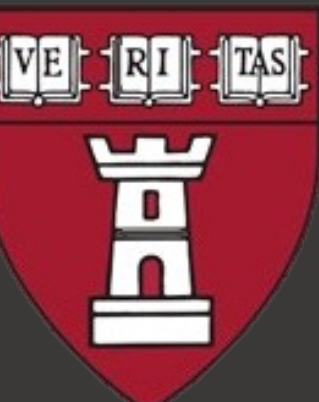




Masticatory Function and Cognitive Health: Investigating a Molecular Connection Between Oral Tissues and the Brain

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BACKGROUND

Amongst the rapidly growing elderly population, there is increasing need for comprehensive prosthodontic treatment to address impaired masticatory function of many etiologies. Compromised oral health may contribute to a myriad of systemic pathologies, including cognitive decline. Several studies have observed an association between poor masticatory function in elderly patients and impaired cognitive function. Previously, we identified a novel retrograde transport mechanism of exosomal neprilysin, a neuroprotective enzyme, from the masseter muscle to the hippocampus through the trigeminal nerve.

Building on our previous studies suggesting that craniofacial tissue-derived exosomes may be delivered to the brain via retrograde axonal transport through cranial nerve junctions, we aim to further investigate this potential pathway. Specifically, this study will compare neurotrophic gene and protein expressions and their exosomal localizations between cell cultures of oral tissue and brain origins. Recent research has highlighted the importance of extracellular vesicles (EVs), particularly exosomes, in intercellular communication within the nervous system. Exosomes can carry a variety of cargo, including proteins, lipids, and nucleic acids, and have been shown to play roles in neuronal development, synaptic function, and neurological disorders.

MATERIALS AND METHODS

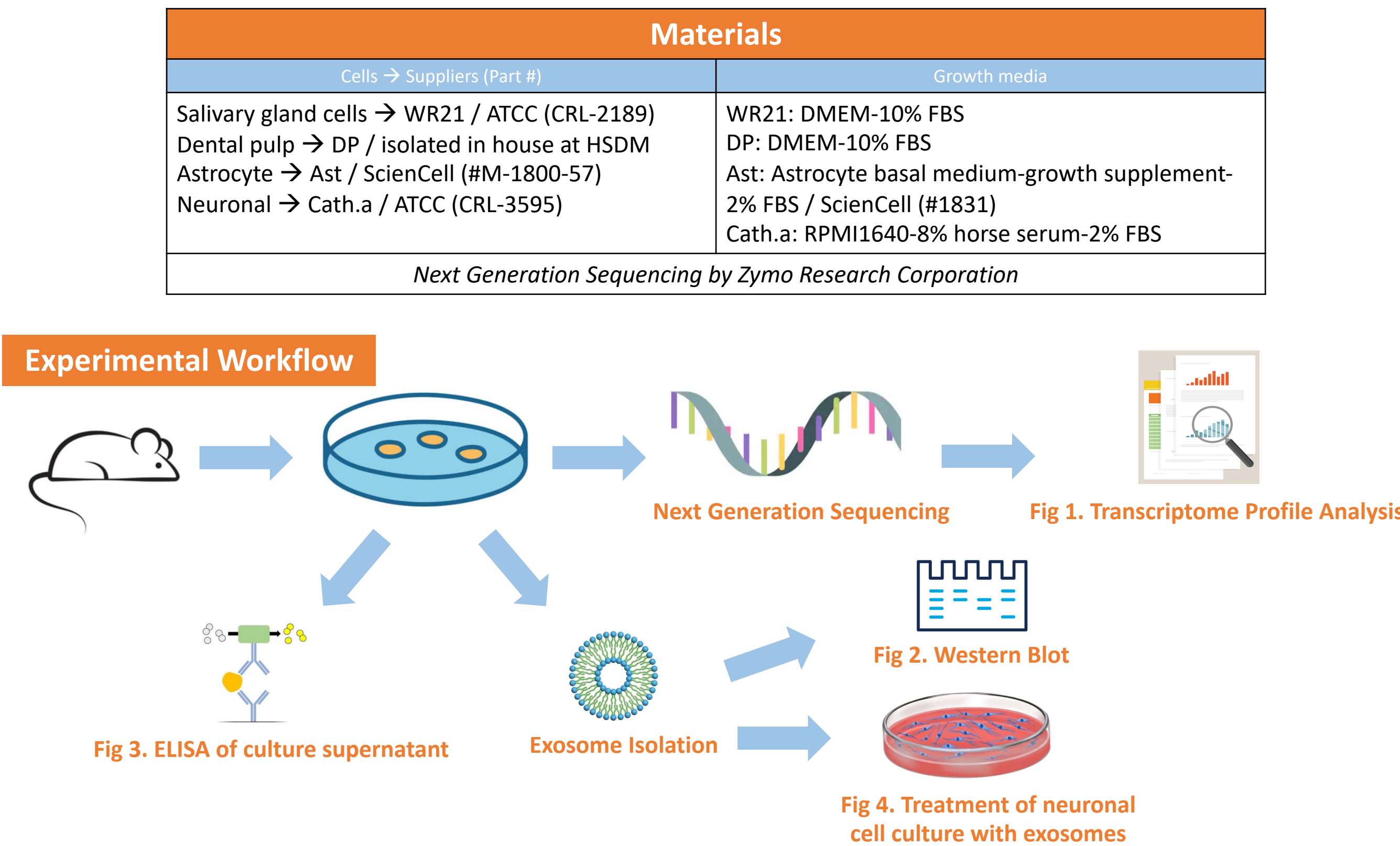


Fig 1. mRNA expression profiles of murine cells from submandibular-glands (WR21), dental-pulp (DP), brain-astrocytes (Ast), and neuronal cell line (CATHa) were obtained using next-generation-sequencing, which was outsourced to Zymo Research Corporation. Gene transcripts were screened for neurotrophic factors with differential expression levels in each cell types and compared. The fold change of neurotrophic gene transcripts between WR21, DP and Cath.a cells. NGS transcripts from astrocytes were screened for neurotrophic genes that were not expressed by Cath.a cells.

Fig 2. and 3. Brain-derived neurotrophic factor (BDNF) levels in exosomes and cell culture supernatant were analyzed via Western blot and ELISA, respectively. BDNF primary antibody mouse anti-BDNF (Abcam / ab108319), CD81 primary antibody, CD81 primary Ab: mouse anti-CD81 (Abcam / ab79559), secondary antibody: IRDye 800cw goat anti-mouse IgG (Li Cor / 926-32350). 10µg of total protein were loaded per lane.

Fig 4. The effect of oral tissue derived exosomes on the growth of Cath.a cells were tested by seeding 5000 Cath.a cells per 100µL in a 96 well plate. Exosomes were supplemented at 20µg total protein/mL in Cath.a growth medium. Cell growth was evaluated by Cell Titer Glow total cellular ATP assay (Promega) at 48h after exosome supplementation

RESULTS

Fig 1. Comparative neurotrophic mRNA transcript analysis between oral tissue and neuronal cells

Submandibular gland and dental pulp cells produce significantly higher levels of neurotrophic molecules in comparison to neuronal cells. They also exhibit equal or greater expression of neurotrophic molecules compared to astrocytes.

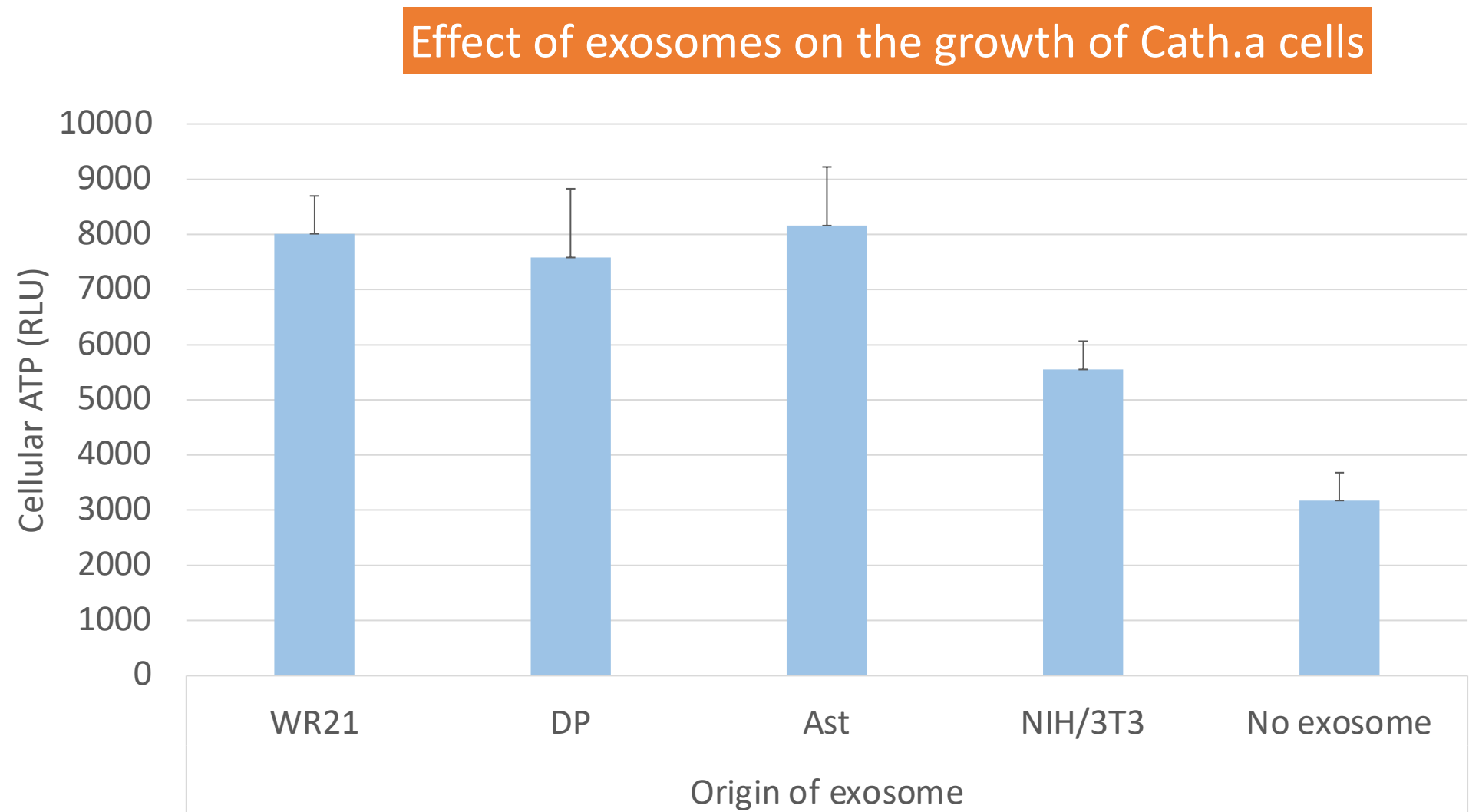
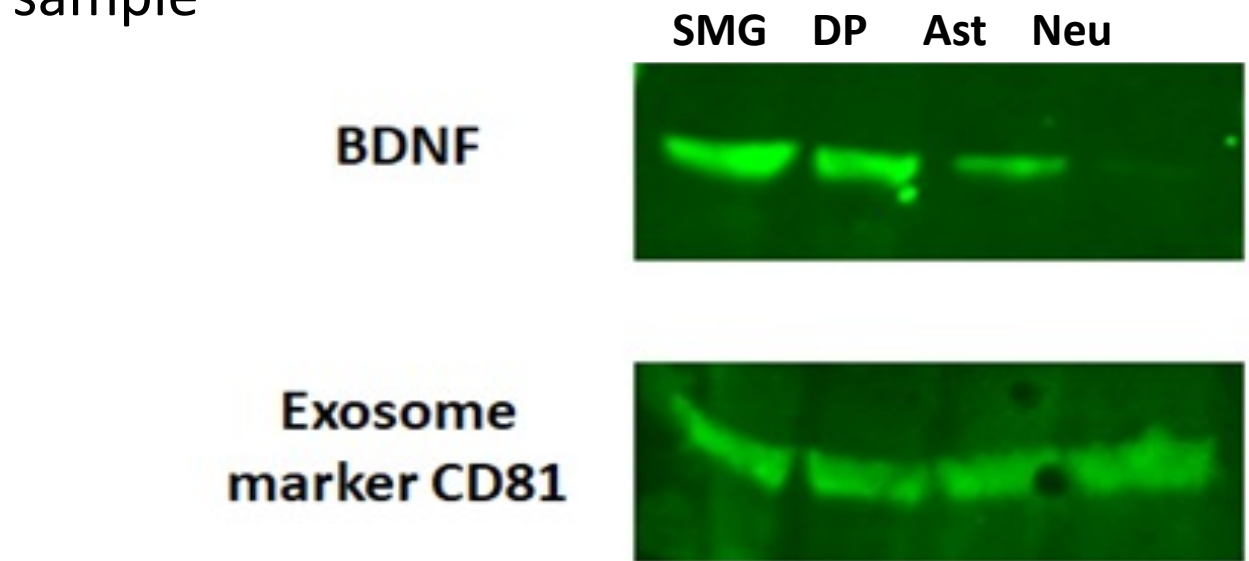
Submandibular gland cells vs Neurons		
Gene	Fold change	padj** < 0.01
Bdnf	28	**
Epha1	62	**
Fgf10	1246	**
Fgf2	386	**
Gdnf	452	**
Gfra1	211	**
Mme	214	**
Nfe2l2	12	**
Ngf	949	**
Ntn1	247	**
Ntn4	33	**
Sema3a	732	**
Sema3b	31	**
Sema3e	148	**
Sema6d	36	**
Sema7a	276	**

Submandibular gland cells vs Astrocytes		
Gene	Fold change	padj** < 0.01
Igf1r	0.9	NS
Atm	2.2	**
Efna1	31.1	**
Ephb4	0.8	NS
Foxo1	0.6	NS
Fus	2.5	**
Nrg1	14.9	**
Nrg2	1.0	NS
Ret	1.4	NS
Sema3f	0.6	NS
Sema7a	21.5	**
Sirt1	3.0	**
Slit2	0.6	NS
Vcp	2.0	**
Vegfa	5.8	**

Fig 2. Western blot analysis of brain-derived neurotrophic factor level in exosomes

a) Exosomes from submandibular gland cells (SMG) and dental pulp cells (DP) contained higher quantities of brain-derived neurotrophic factor compared to astrocytes and neuronal cells.

b) Control to verify equal quantity of exosomes from each sample



Dental pulp cells vs Neurons		
Gene	Fold change	padj** < 0.01
Bdnf	31	**
Epha1	2303	**
Epha3	2143	**
Epha4	15	**
Fgf10	2218	**
Fgf7	2896	**
Gdnf	894	**
Gfra1	32	**
Mme	1340	**
Nfe2l2	11	**
Ngf	3405	**
Nrg1	11	**
Ntn1	407	**
Ntn4	33	**
Ntn2	109	**
Parp3	51	**
Sema3a	54	**
Sema3b	29	**
Sema3c	21	**
Sema3d	7131	**
Sema3e	127	**
Sema6d	19	**
Sema7a	36	**
Slitrk6	84	**
Vegfc	417	**
Vegfd	32	**

Dental pulp cells vs Astrocytes		
Gene	Fold change	padj** < 0.01
Atm	1.0	NS
Efna1	2	NS
Efna2	0.4	NS
Efna4	0.4	NS
Fus	1.1	NS
Igf1r	0.7	NS
Nipsnap1	1.4	NS
Ret	0.5	NS
Robo1	0.7	NS
Sirt1	0.8	NS
Sirt2	0.8	NS
Sirt3	1.1	NS
Sirt4	0.7	NS
Sirt5	0.6	NS
Sirt6	1.1	NS
Sirt7	2.1	**
Snap23	3.3	NS
Snap29	1.2	NS
Snap47	1.0	NS
Snapc1	1.9	NS
Snapin	0.6	NS
Vcp	2.9	**

Fig 3. Enzyme-Linked Immunosorbent Assay (ELISA) of BDNF

Expression levels of BDNF within the culture supernatant of astrocytes far exceeded that of submandibular gland cells, dental pulp cells, and Cath.a cells

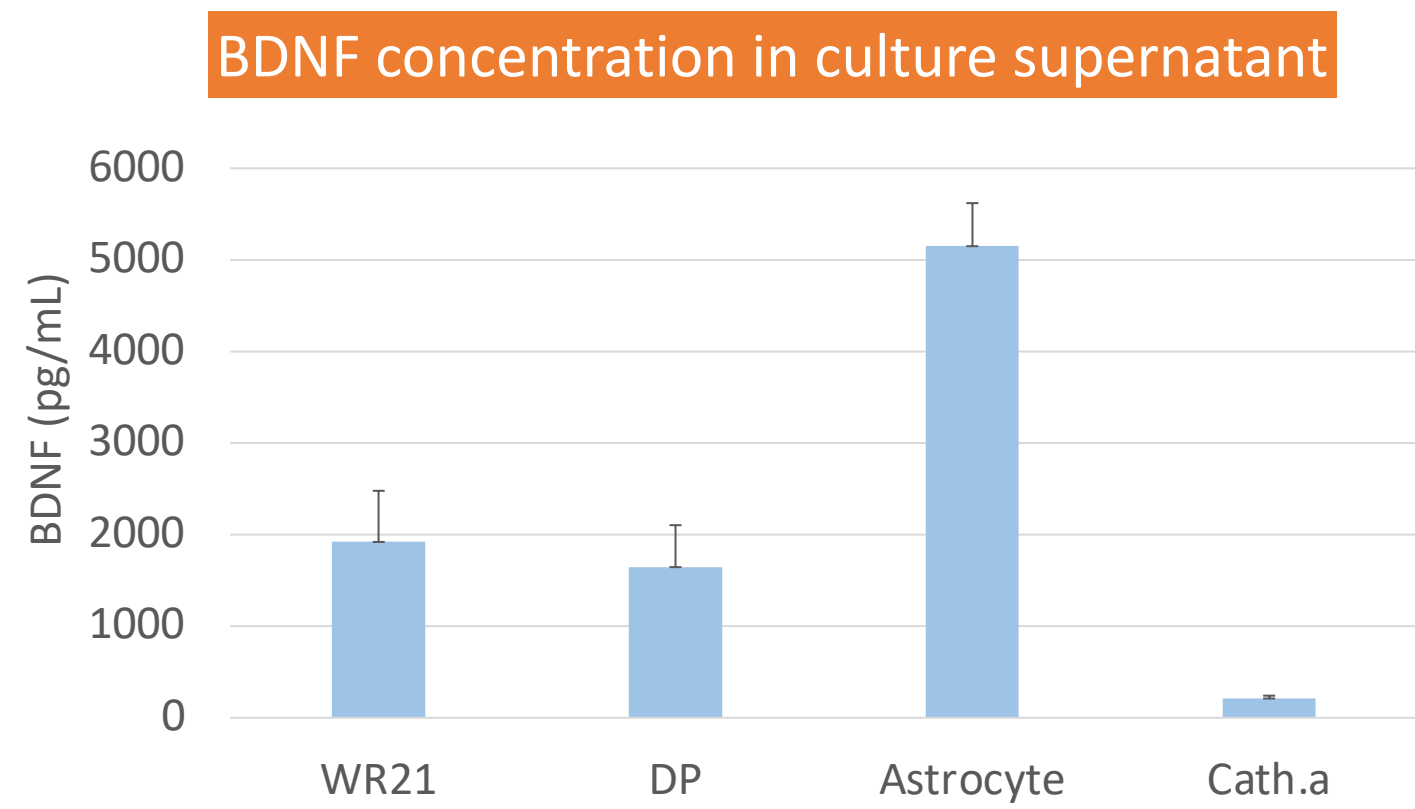


Fig 4. Effect of exosomal cargo on the growth of Cath.a cells

Cultured Cath.a cells treated with exosomes from submandibular gland cells (SMG), dental pulp cells (DP), and astrocytes (Ast) exhibit greater cellular ATP levels compared to un-treated cultured Cath.a cells.

RESULTS

Submandibular gland cells and dental pulp cells expressed a broad array of neurotrophic genes that were lacking in neuronal cell expression, despite their own lack of need for these neurotrophic proteins. This further aids our initial findings that craniofacial and oral tissues secrete molecules that travel to the brain via retrograde axonal transport to the aid of neuronal cells. Brain-derived neurotrophic factor, which was selected as a representative neurotrophic molecule, is packaged into exosomes by submandibular gland cells and dental pulp cells, while BDNF is secreted directly into supernatant of astrocytes. Exosomes are able to pass through the blood brain barrier, while free floating proteins are not, which further suggests that oral tissue cells are packaging neurotrophic molecules into exosomes for the purpose of transport into the brain. When cultured with the contents or oral tissue cell derived exosomes, Cath.a cells exhibited enhanced growth, indicated that the contents of exosomes released by oral tissue cells contain molecules that are beneficial to the growth of neuronal cells.

CONCLUSIONS

We can conclude that there is an intimate and complex relationship between oral tissues and the brain at the molecular level. The findings from this study suggest that oral tissues may play a crucial role in supplying essential biomolecules to the brain. Results from this ongoing study further highlight the impact of masticatory function and oral health on systemic health and provides hope for development of therapeutics.

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