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Systematic Review

The Effect of the Dental Stem Cell Secretome on Tissue Regeneration: A Systematic Review

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ABSTRACT

Secretome therapy is a promising approach in tissue regeneration because it can reproduce most of the advantages of cell-based therapies. This review aims to investigate the most prominent effect of using dental-derived secretome on tissue regeneration using a systematic review approach. A systematic electronic search was conducted via the PubMed, Scopus and Wiley online library databases for studies published in English up to October 2020. All the articles from the databases were screened, and the criteria for inclusion and exclusion were applied. Forty papers were included in the study, whereby there were 16 *in vitro* studies and 11 *in vivo* studies with different animal models. No clinical trial has been reported yet. The most studied DSCs were human SHEDs (12 studies), followed by human DPSCs (11) and human PDLSCs (5). The majority of the studies used secretome from human SHEDs and DPSCs. TGF-β1 is the most frequently detected protein in the secretome, which comes from six types of DSCs, followed by NGF and NT-3, which were found in five different types of DSC secretome. The compositions of the secretome were found to promote the regeneration of the tissues through their neurogenic, angiogenic, osteogenic and odontogenic effects, with the majority of studies reviewed reporting using them for nondental tissue regeneration. From this review, DSC-CM reported favorable tissue regeneration potential; however, many factors need to be explored in future research with regard to the application of secretome delivery, particularly DSC-CM, in the clinical setting.

Key words: dental-derived secretomes, paracrine mediated therapy, tissue regeneration

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INTRODUCTION

In regenerative medicine, tissue engineering has arisen as a promising approach for the restoration, repair and healing of organ and tissue functions, especially in tissues susceptible to disease, injury, and degeneration. At present, tissue engineering studies are focused on adult mesenchymal stem cells, which have become among the most frequently explored types of cells in this field[1]. Mesenchymal stem cells (MSCs) are undifferentiated multipotent cells that possess self-renewal capabilities and can differentiate into various mesoderm cell lineages, such as osteogenic, adipogenic and chondrogenic lines. Aside from bone marrow and adipose tissue, cells possessing stem cell characteristics have also been successfully isolated from different parts of the tooth. These isolated cells are known as dental stem cells and include dental pulp stem cells (DPSCs)[2,3], stem cells from exfoliated deciduous teeth (SHED)[4], periodontal ligament stem cells (PDLSCs)[5], stem cells from apical papilla (SCAP)[6,7], dental follicle progenitor cells (DFCs)[8] and gingival tissues derived from mesenchymal stem cells (GMSCs)[9,10].

The majority of dental-derived stem cells (DSCs) can be obtained from human exfoliated deciduous teeth and orthodontically extracted premolars and third molars. These sources of stem cells are considered biological waste in dentistry despite containing multipotent stem cells[4,11]. Premolars are the most common teeth indicated for extraction in orthodontics and are often ideal for the relief of anterior and posterior crowding. However, the decision of extracting between the first or second premolars depends on several factors, including the anchorage requirement, the severity of crowding, and the amount of overbite and overjet[12]. Extraction of the third molar for orthodontic reasons is rare, as both orthodontists and oral surgeons must make appropriate decisions and consider the potential risks and benefits of the procedure specifically pertaining to the surgical removal of asymptomatic impacted third molars[13]. There are several orthodontic reasons for the extraction of third molars that aim to prevent late mandibular incisor crowding[14], to allow molar distalization[15] and to prepare for orthognathic surgery[16].

Experimental research using stem cells showed that they produce reliable and effective tissue regenera-

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tion. However, there are several shortcomings in terms of the clinical application of therapy using MSCs[17]: (i) the host immune response might cause rejection of transplanted MSCs in the long term, (ii) there could be an imbalance and disturbance in the homeostasis of local tissue, (iii) the risk for tumor formation may increase due to long-term *ex vivo* expansion and/or due to immunosuppression of the local tissue, and (iv) the chances for ectopic tissue formation by the transplanted MSCs become higher[13].

To overcome these drawbacks of using MSCs, an increasing number of studies have reported the use of the MSC secretome[18]. Secretome are various cellular products, such as cytokines, growth factors and enzymes secreted by MSCs[19,20]. These biologically active chemical products play significant roles in diverse aspects of tissue function, repair and homeostasis[21,22]. The function of the secretome is to suppress immune responses[23], reduce oxidative stress[13], stimulate angiogenesis[24], and induce the recruitment, proliferation and differentiation of endogenous stem cells[25]. Secretome are easily obtained via *in vitro* culture of various cells, where they are contained or extracted from the culture media, often referred to as conditioned media (CM). In various studies, CM from stem cells has shown promising results in regenerative medicine and tissue engineering[26], including CM from SHED and CM from healthy and inflamed periodontal tissue[27,28].

When compared to stem cell therapy, secretome therapy offers potential benefits because it can reproduce most of the advantages of cell-based therapies with minimal side effects. Furthermore, using the secretome as therapy provides many practical advantages, such as ease of preservation, sterilization, and packaging[1]. Apart from that, they also have an extended duration of storage without losing their properties[29,30], ease in gauging proper dosages and can be produced in mass[20] as well as a noninvasive extraction procedure that may save both time and cost of production[24]. Despite the potential benefits of the secretome, the effect of the dental-derived secretome on specific types of tissue has not been systematically reviewed. The focus question is to systematically review *in vitro*, *in vivo* and clinical studies on the effect of the dental-derived secretome in terms of tissue regeneration potential. Thus, we aim to identify the most prominent effect of using a dental-derived secretome on tissue regeneration using a systematic review approach.

METHODS

The Preferred Reporting Items for Systematic Review (PRISMA) checklist was used as a guideline in conducting this review[31]. A comprehensive and systematic electronic search of MEDLINE via PubMed, Scopus and Wiley online library databases was conducted for studies published in English up to October 2020 (**Figure 1**).

Study design

This study systematically reviewed and summarized all the published studies regarding the use of dental stem cell-derived conditioned media in tissue regeneration by searching electronic databases.

Inclusion criteria

The included studies were *in vivo, in vitro* and clinical studies on the dental stem cell secretome and tissue regeneration. Language was limited to English, and no manual or gray literature search was included.

Exclusion criteria

All studies on embryonic stem cells, bone marrow stem cells, induced pluripotent stem cells, other types of mesenchymal stem cells, cell therapy, secretome derived from ameloblasts, odontoblasts, dental epithelium, narrative reviews, systematic reviews and/or meta-analyses.

Information sources and search strategies

A comprehensive search of online databases was implemented through MEDLINE via PubMed, SCOPUS and Wiley Online Library. All studies published up to October 2020 were included. A Boolean operator was used for the search strategy by combining terms and free text words: "dental stem cells" AND secretome, "dental secretome" OR "paracrine therapy" AND regeneration, "dental stem cells" AND "conditioned medium" AND regeneration, dental AND "paracrine mediated therapy". Then, all duplicate papers were removed by the reference manager software (Mendeley).

Study selection

The data were extracted independently by four authors (N.S. N, M.M. D, F. and M.H. with an extraction form specifically designed for this study. Then, any disagreement on the data extracted was resolved through discussion and mutual consensus between the authors. Interrater reliabilities were calculated using Cohen's Kappa (κ = 0.704, which indicates substantial agreement).

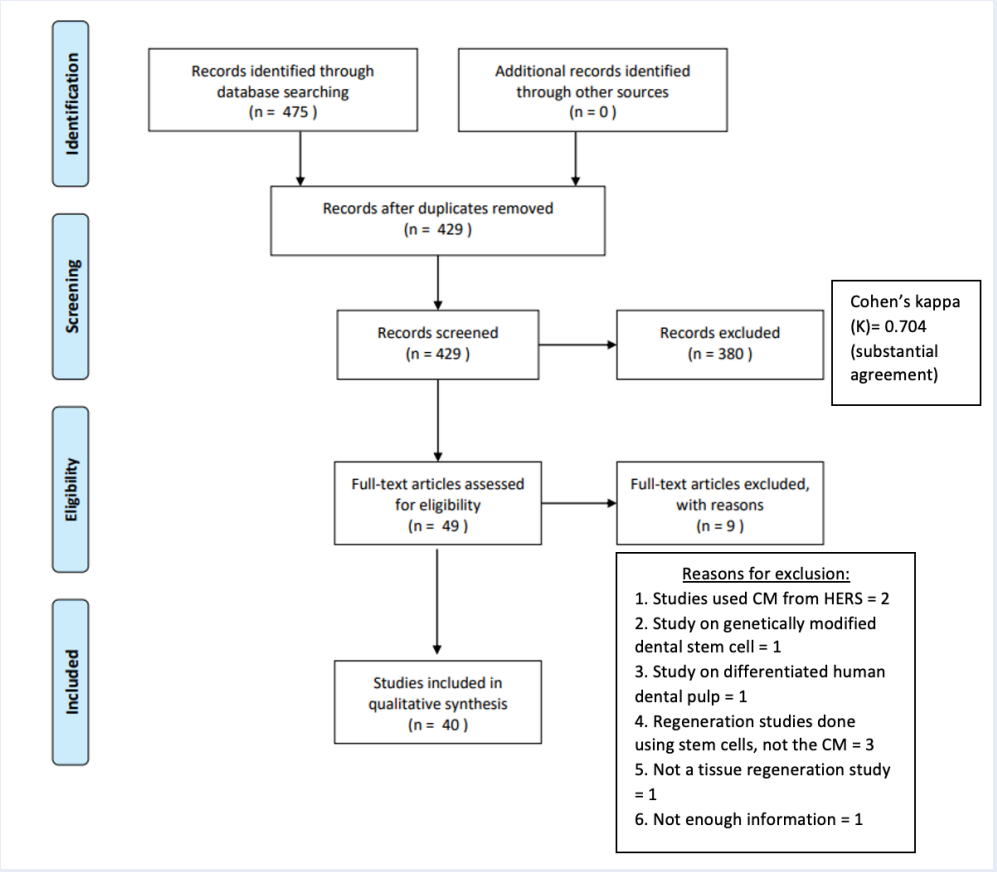

Figure 1: Flow diagram for the papers selection.

Data collection process

For data collection, necessary information was extracted as follows: the characteristics of the study (authors, year of publication, country of the study conducted), type of study design (*in vitro*, *in vivo*, clinical studies), source of dental stem cell secretome, test for dental stem cell markers, test and growth factors identified from the secretome, test and analysis for regeneration with the findings, clinical test used, and any biomaterials used for regeneration.

RESULTS

Study overview

A total of 40 studies were selected after thorough screening by the authors, as listed in **Table 1**. Of these, 16 are *in vitro* studies, and 11 are *in vivo* studies with various types of animal models. The remaining 13 studies were combinations of *in vitro* and *in vivo* intervention studies. There were no clinical trials reported. Nine studies were excluded because the studies used CM from Hertwig's epithelial root sheath (HERS) (2), study on genetically modified dental stem cells (1), study on differentiated human dental pulp (1), regeneration studies done using stem cells not the CM (3), not a tissue regeneration study (1), and insufficient information (1).

The source of dental-derived stem cell secretome is from either humans or animals. There were 29 studies that used human dental stem cells as the source of the conditioned media, while nine studies used animal dental stem cells, such as stem cells from rats, pigs and dogs. One study used both dental stem cells from humans and porcines, whereby they utilized tooth germ cells. Another study did not specify the source of tooth germ cells used.

The source of the secretome used in all the studies varies from dental pulp stem cells (DPSCs), human exfoliated deciduous teeth (SHEDs), stem cells from apical papilla (SCAPs), dental follicle stem cells (DFSCs), periodontal ligament stem cells (PDLSCs), gingival mesenchymal cells (GCMs) and tooth germ cells (TGCs). TGCs can be further subcategorized into apical tooth germ cells (APTGCs), embryonic tooth

Table 1: Summary of studies selected

Year (Location)	Study setting	Secretome cellular source (Organism)	Stem cell marker reported (Technique)	Secretome proteomic analysis (Technique)	Effects (details)					Reference
					Angiogenic	Neurogenic	Osteogenic	Odontogenic	Others	
2009 (China)	*In vitro and in vivo*	APTGC (rat)	Yes (FC)	No					x (cementogenic)	[32]
2010 (USA)	*In vitro*	DPSC (human)	No	No				x		[33]
2010 (China)	*In vitro and in vivo*	ETGC (rat)	No	No			x	x		[34]
2011 (China)	*In vitro*	DFC (rat)	No	No			x			[35]
2011 (China)	*In vitro and in vivo*	TGC (human and porcine)	No	No				x		[36]
2013 (Japan)	*In vitro*	DPSC (human)	Yes (FC)	No					x (anti-inflammatory, anti-apoptotic)	[37]
2014 (China)	*In vitro*	DFC (rat)	No	No			x			[38]
2015 (Japan)	*In vitro and in vivo*	DPSC (porcine)	No (referred previous work)	Yes (WB)				x		[39]
2015 (Japan)	*In vivo*	SHED (human)	Yes (FC)	Yes (multiplex assay)					x (anti-apoptosis, anti-inflammatory)	[40]

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Table 1 continued

Year (Location)	Study setting	Secretome cellular source (Organism)	Stem cell marker reported (Technique)	Secretome proteomic analysis (Technique)	Effects (details)					Reference
					Angiogenic	Neurogenic	Osteogenic	Odontogenic	Others	
2015 (Japan)	*In vivo*	SHED (human)	No	Yes (WB)					x (anti-inflammatory)	41
2015 (Japan)	*In vitro*	DPSC (dog)	Yes (FC)	No	x	x	x		x (anti-apoptotic, chemotactic)	42
2015 (Japan)	*In vitro and in vivo*	SHED (human)	No	Yes (ELISA)	x	x				43
2015 (Japan)	*In vitro and in vivo*	SHED (human)	No	No					x (anti-inflammatory)	40
2016 (Japan)	*In vitro and in vivo*	DPSC (porcine)	No	No	x			x		44
2016 (Japan)	*In vivo*	PDLSC (human)	No	Yes (LC/MS)			x			45
2016 (Japan)	*In vitro*	DPSC (human)	No	No		x			x (chemotactic, proliferative)	46
2017 (Norway)	*In vitro and in vivo*	DPSC (human; normoxic and hypoxic)	No (referred previous work)	Yes (ELISA)	x		x			47

Continued on next page

Table 1 continued

Year (Location)	Study setting	Secretome cellular source (Organism)	Stem cell marker reported (Technique)	Secretome proteomic analysis (Technique)	Effects (details)					Reference
					Angiogenic	Neurogenic	Osteogenic	Odontogenic	Others	
2017 (Italy)	*In vitro and in vivo*	PDLSC (human; hypoxic)	No	Yes (WB)					x (anti-inflammatory)	[48]
2017 (Japan)	*In vivo*	SHED (human)	No (referred previous work)	Yes (ELISA)	x	x			x (anti-inflammatory)	[49]
2017 (Sweden)	*In vitro and in vivo*	SCAP, DPSC, PDLSC (all human)	Yes (FC)	Yes (ELISA)		x				[50]
2017 (India)	*In vitro*	SCAP, DPSC, DFC (all human)	Yes (FC)	Yes (multiplex ELISA)		x				[51]
2017 (Japan)	*In vitro*	DPSC (human)	Yes (FC)	No		x			x (chemotactic, anti-inflammatory, anti-apoptosis)	[52]
2017 (Italy)	*In vitro*	GMSC (human)	Yes (FC)	Yes (WB)		x			x (anti-inflammatory)	[53]

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Table 1 continued

Year (Location)	Study setting	Secretome cellular source (Organism)	Stem cell marker reported (Technique)	Secretome proteomic analysis (Technique)	Effects (details)					Reference
					Angiogenic	Neurogenic	Osteogenic	Odontogenic	Others	
2018 (Norway)	*In vitro*	DPSC (human)	No (referred previous work)	Yes (ELISA)	x					54
2018 (Japan)	*In vivo*	SHED (human)	No	No		x				55
2019 (Taiwan)	*In vivo*	DPSC (rat)	Yes (FC)	Yes (multiplex ELISA)					x (anti-inflammatory)	56
2019 (Brazil)	*In vitro and in vivo*	SHED (human)	Yes (FC)	Yes (ELISA)	x			x	x (wound closure)	57
2019 (Belgium)	*In vitro*	DPSC (human)	No (referred previous work)	No		x			x (cell migration)	58
2019 (Japan)	*In vitro and in vivo*	SHED (human)	No	Yes (ELISA)		x				59
2020 (Iran)	*In vitro*	PDLSC (human)	Yes (FC)	No					x (SC proliferation)	60
2020 (Taiwan)	*In vivo*	SHED (human)	No	Yes (multiplex ELISA)		x (neuro-protective)			x (anti-inflammatory)	61

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Table 1 continued

Year (Location)	Study setting	Secretome cellular source (Organism)	Stem cell marker reported (Technique)	Secretome proteomic analysis (Technique)	Effects (details)					Reference
					Angiogenic	Neurogenic	Osteogenic	Odontogenic	Others	
2020 (Japan)	*In vivo*	SHED (human)	No	Yes (multiplex assay)			x			62
2020 (China)	*In vitro and in vivo*	DFSC (rat)	Yes (FC)	No				x	x (anti-apoptotic, anti-inflammatory)	63
2020 (Belgium)	*In vitro*	DPSC (human)	No	No					x (chondrogenic)	11
2020 (Malaysia)	*In vitro*	SHED (human)	Yes (FC)	Yes (ELISA)					x (anti-inflammatory, chondrogenic)	64
2020 (Japan)	*In vivo*	SHED (human)	No	Yes (LC/MS)			x (osteoclast inhibition)		x (anti-apoptotic, anti-inflammatory)	65
2020 (China)	*In vivo*	GMSC, PDLSC (both human)	Yes (FC)	No			x			66
2020 (Japan)	*In vivo*	DPSC (human)	No	No					x (cell proliferation)	**67**
2020 (China)	*In vitro*	SCAP (human)	No	Yes (WB)			x	x		**68**

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Table 1 continued

Year (Location)	Study setting	Secretome cellular source (Organism)	Stem cell marker reported (Technique)	Secretome proteomic analysis (Technique)	Effects (details)					Reference
					Angiogenic	Neurogenic	Osteogenic	Odontogenic	Others	
2019 (China)	*In vitro*	TGC (unknown organism)	No	No			x	x		[69]

ELISA: enzyme-linked immunosorbent assay; FC: flow cytometry; WB: Western blot

germ cells (ETGCs) and neonatal tooth germ cells (NTGCs). Human SHEDs are the most studied DSCs (12 studies), followed by human DPSCs (11) and human PDLSCs (5). Secretome from human SHEDs and DPSCs contributed to the majority of the studies.

To ensure that the secretome obtained is of stem cell origin, most studies would need to show the presence of stem cell markers on the cells used. However, from this review, there were only 18 studies reporting on stem cell markers, whereas the remaining studies did not. Among studies that reported stem cell markers, 15 used flow cytometry, while the remaining studies did not state the technique used.

Proteomic analysis of the DSC secretome

Sixteen studies reported proteomic analysis of CM. The techniques used in identifying the secreted factors from the DSCs are ELISA (8 studies), Western blot (1), multiplex analysis (5) and liquid chromatography tandem mass spectrometry (LC–MS/MS) (2). The most frequently detected protein is transforming growth factor-β1 (TGF-β1), which is present in the secretome of six types of DSCs: human SHEDs, SCAPs, DPSCs, DFCs, and GMSCs as well as rat DPSCs. This was followed by nerve growth factor (NGF) and neurotrophin-3 (NT-3), which were found in five different types of DSC secretome. Brain-derived neurotrophic factor (BDNF), tissue inhibitor of metalloproteinase-1 (TIMP-1) and vascular endothelial growth factor (VEGF) were detected in the secretome from four different sets of DSCs. Further details of the proteins detected in the DSC secretome are presented in **Table 2**.

Table 2: List of frequently reported protein contents of the DSC secretome

Protein	Source of secretome	Reference
TGF-β1	Human SHEDs (2)	Muhammad et al 2020 [64], Chen et al 2020 [61]
	Human SCAPs (1)	Kumar et al 2016 [51]
	Human DPSCs (1)	Kumar et al 2016 [51]
	Human DFCs (1)	Kumar et al 2016 [51]
	Human GMSCs (1)	Rajan et al 2017 [53]
	Rat DPSCs (1)	Chen et al 2019 [56]
NGF	Human SHEDs (2)	Miura-Yura et al 2019 [59], Sugimura et al 2015, [43]
	Human SCAPs (1)	Kumar et al 2016 [51]
	Human DPSCs (1)	Kumar et al 2016 [51]
	Human DFCs (1)	Kumar et al 2016 [51]
	Human GMSCs (1)	Rajan et al 2017 [53]
NT-3	Human SHEDs (2)	Sugimura et al 2015, [43] Hiraki et al, 2020 [62]
	Human SCAPs (1)	Kumar et al 2016 [51]
	Human DPSCs (1)	Kumar et al 2016 [51]
	Human DFCs (1)	Kumar et al 2016 [51]
	Human GMSCs (1)	Rajan et al 2017 [53]
BDNF	Human SCAPs (2)	Kolar et al 2017 [50], Kumar et al 2016 [51]
	Human DPSCs (2)	Kolar et al 2017 [50], Kumar et al 2016 [51]
	Human SHEDs (3)	Miura-Yura et al 2019 [59], Sugimura et al 2015, [43] Hiraki et al 2020 [62]
	Human DFCs (1)	Kumar et al 2016 [51]
TIMP_1	Human DPSCs (1)	Gharaei et al 2018 [54]
	Human SHEDs (2)	Chen et al 2020 [61], Hiraki et al 2020 [62]
	Human PDLSCs (1)	Nagata 2016 [45]
	Rat DPSCs (1)	Chen et al 2019 [56]

Continued on next page

Table 2 continued

Protein	Source ofsecretome	Reference
VEGF	Human SHEDs (4)	de Cara et al 2019[57], Miura-Yura et al 2019[59], Sugimura et al 2015,[43] Hiraki et al, 2020[62]
	Human DPSCs (3)	
	Human SCAPs, (1)	Gharaei et al 2018[54], Kolar et al, 2017, Fujio et al 2015[47]
	Human PDLSCs (2)	Kolar et al 2017[50]
		Kolar et al 2017[50], Nagata, 2016[45]
GCSF	Human SCAPs (1)	Kumar et al 2016[51]
	Human DPSCs (1)	Kumar et al 2016[51]
	Human DFCs (1)	Kumar et al 2016[51]
IFN-gamma	Human SCAPs (1)	Kumar et al 2016[51]
	Human DPSCs (1)	Kumar et al 2016[51]
	Human DFCs (1)	Kumar et al 2016[51]
IGF_BP2	Human DPSCs (1)	Gharaei et al 2018[54]
	Human SHEDs (1)	Chen et al, 2020[61]
	Human PDLSCs (1)	Nagata, 2016[45]
IGF-BP3	Human DPSCs (1)	Gharaei et al 2018[54]
	Human SHEDs (1)	Chen et al, 2020[61]
TIMP-2	Rat DPSCs (1)	Chen et al, 2019[56]
	Human SHEDs (1)	Chen et al, 2020[61]
Angiopontin-2	Human DPSCs (1)	Fujio et al 2015[47]
BMP-2	Human SHEDs (1)	Hiraki et al, 2020[62]
BMP-4	Human SHEDs (1)	Hiraki et al, 2020[62]
BMP-5	Human SHEDs (1)	Chen et al, 2020[61]
CNTF	Human SHEDs (1)	Sugimura et al 2015[43]
FGF-2	Human SHEDs (2)	Miura-Yura et al 2019[59], Hiraki et al, 2020[62]
GDNF	Human SHEDs (2)	Sugimura et al 2015,[43] Hiraki et al, 2020[62]
HGF	Human SHEDs (2)	Sugimura et al 2015,[43] Hiraki et al, 2020[62]
IGF-1	Rat DPSCs (1)	Chen et al, 2019[56]
IGF-BP4	Human SHEDs (1)	Chen et al, 2020[61]

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Table 2 continued

Protein	Source of secretome	Reference
IGF-BP6	Human SHEDs (1)	Chen et al, 2020[61]
	Human PDLSCs (1)	Nagata, 2016[45]
IL-10	Human SHEDs (1)	Muhammad et al 2020[64]
	Human GMSCs (1)	Rajan et al, 2017[53]
IL-6	Human SHEDs (1)	Muhammad et al 2020[64]
MCP-1	Human SHEDs (2)	Kano et al 2016[49], Hiraki et al, 2020[62]
	Human PDLSCs (1)	Nagata, 2016[45]
PAI-1	Human DPSCs (1)	Gharaei et al 2018[54]
PDGFR-B	Human PDLSCs (1)	Nagata, 2016[45]
SDF-1	Human DPSCs (1)	Gharaei et al 2018[54]

Functional analysis using the DSC secretome

Overview

Despite using DSCs to obtain the secretome, the majority of studies we reviewed reported using the cells for nondental applications, with neuroregeneration being the most frequently studied (12 out of 40 studies). Others include regeneration of bone tissue, blood vessels, salivary duct, lung, and liver. Among the studies reporting using secretome for dental tissue regeneration (15 studies), three areas were examined: (i) dental pulp, (ii) periodontal, and (iii) dentin. The effects of the secretome are summarized below.

Neurogenic effects

Among studies reporting favorable neurogenic effects (total of 15), all but two used the DSC secretome obtained from humans instead of animal cells. Most of the studies using the human DSC secretome were sourced from SHED, followed by secretome from DPSCs, PDLSCs, SCAP, and GMSCs. When looking from the experimental design perspective, most of the neurogenic studies were performed in both cell culture and animal models of various neurodegenerative diseases (such as hemorrhagic stroke, Alzheimer's disease or diabetic neuropathy), with only six studies reporting the effects of cell culture experiments exclusively. The most frequently reported *in vitro* finding is enhanced neurite outgrowth, followed by increased neuronal proliferation. Three out of eight studies reporting neurite outgrowth also reported increased expression of BDNF with secretome treatment.

Angiogenic effects

Most of the data on the angiogenic effects of the DSC secretome were obtained from work on human umbilical vein endothelial cells (HUVECs), with only one from human dermal microvascular endothelial cells. From a total of nine studies, four of them reported only *in vitro* findings, whereas the remainder also reported effects in animal models. The secretome were obtained from either DPSCs (6 studies; 4 of which were human cells) or SHED (all 3 from humans). Looking at the *in vitro* studies, five of them reported enhanced vascular network formation, three studies reported increased cell proliferation/viability, while two reported observing HUVECs differentiating into VE-cadherin-positive endothelial cells.

Osteogenic/odontogenic effects

Odontogenic effects are seen when cellular differentiation indicates that the formation of odontoblasts is imminent. Odontoblasts are cells that lay down the dentin layer during tooth formation and are usually found in the dental pulp. During in vitro experimentation, Alizarin red staining used to detect intracellular mineralization was used to detect odontogenic or osteogenic processes. In our review, we found 12 studies looking at the osteogenic/odontogenic effects of the secretome, of which only four were sourced from humans, while the rest were obtained from rats and pigs. There were multiple cell types from which the secretome were obtained, with TGCs being the most used (in four studies), followed by DPSCs, DFSCs (three each), SCAP and SHED. In determining the osteogenic/odontogenic effects of the secretome, the parameters reported were enhanced intracellular mineralization (6 studies), increased expression of mineralization markers such as ALP, BSP and CAP (5), and increased odontoblastic differentiation (4). The single study that used the SHED secretome reported inhibition of osteoclastic activity *in vitro*, which was reflected as a reduction in bone resorption under radiography *in vivo*.

DISCUSSION

Most of the studies reviewed here were preclinical *in vitro* and animal studies reporting possible mechanisms of secretome effects, which can be grouped into neurogenic, angiogenic, osteogenic and odontogenic. When compared to the list of proteins detected in the secretome, a correlation can be observed between the type of protein found and the effects seen. As an example, TGF-ß1 is a mediator of osteogenic differentiation and was repeatedly reported to be a component of secretome from various DSCs. Furthermore, NGF, NT-3 and BDNF are well-known neurotrophic factors[70–72] that correlate with the neuroregenerative effects seen in a large proportion of the studies here.

While there are various sources of the DSC secretome, those obtained from the culture of DSCs acquired from permanent teeth (such as DPSCs and PDLSCs) as well as deciduous teeth (called SHEDs) were mostly reported. This is well explained by the fact that such cells are the most easily accessible for the isolation of stem cells[73]. However, not all studies confirmed their claim that the cells they used are stem cells, as only 19 studies reported performing stem cell marker analysis. Other studies that did not report performing this analysis referred to previous publications in their methodology sections. This step is crucial, as it confirmed the stemness of the cells obtained from the various intraoral sources[74].

At present, clinical studies reporting the effectiveness of the DSC secretome in clinical tissue regeneration

are still absent. Until June 2021, there were only three ongoing clinical trials using the MSC secretome registered with the US National Institute of Health (https://clinicaltrial.gov), with the secretome used in all three studies reported to be from bone marrow MSCs. This could be due to a number of reasons. First, the potential of the DSC secretome to instigate an adverse reaction may not have been well studied. This is reflected in an article search on Scopus using the terms "secretome OR (conditioned media)" AND "toxic*", which returned a result set that was overwhelmingly positive in nature. While this is encouraging, the lack of reports on the propensity of the secretome to cause any adverse biological response will hinder their clinical translation, as the content of the secretome varies and is likely to be antigenic in nature when administered[75].

Hence, the important nonclinical studies that need to be investigated further are on the inflammatory reaction at the local and systemic levels. Although the PDLSC secretome was found to be able to suppress the proinflammatory cytokine expression (tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-1β) of the surrounding healing tissues after 5 days of implantation in an *in vivo* periodontal defect, the findings were not statistically significant compared to the control group[45]. In another study in which the PDLSC secretome was used on the spinal cord of a multiple sclerosis animal model, there was reduced expression of proinflammatory mediators (IL-17 and interferon gamma (IFN-γ))[48]. Other studies that showed immunosuppression of proinflammatory cytokines in animal models include the secretome originating from human SHEDs[40,65,43], human DPSCs[52] and human GMSCs[53]. However, one study showed a contradictory finding that the DPSC secretome caused increased expression of proinflammatory cytokines such as IL-1α, IL-6 and IL-8[39]. To advance knowledge regarding the secretome's biocompatibility, more similar studies are needed to help with efforts to test the secretome in preclinical and clinical human studies.

Another possible reason for the lack of clinical studies would probably be the difficulty in determining the exact preparation of the secretome to be used. The preparation needs to ensure that the contents of the secretome are properly preserved with minimal protein degradation and that it has adequate shelf life. As mentioned in our results section, multiple lines of evidence were found with regard to the composition of the secretome, which includes a vast range of growth factors and cytokines[76].

The results from the proteomic analysis in this review reveal that insulin growth factor-1 (IGF-1) was found only in rat DPSC-CM[56], while no IGF-1 was detected in human DSC-CM. IGF-1 is a small peptide with 70 amino acids and has autocrine, paracrine and endocrine effects. The synthesis of IGF-1 mainly comes from the liver[77], and it is an essential mediator of cell growth, differentiation and transformation[78,79]. The findings from this review are in line with the results from Caseiro et al., who performed a study to compare the profiles of metabolomic and bioactive factors of the human umbilical cord and DPSC secretome[80]. The profiling revealed that no IGF-1 was found from the secretome analysis. The lack of IGF-1 expression in the human stem cell secretome, particularly DSC-CM, might contribute to the binding of this hormone with IGF-binding proteins (IGFBPs). IGFBP expression depends on the tissue site and developmental stage at different concentrations in different body compartments[81]. As shown by the results from this review, IGFBP-2, IGFBP-3, IGFBP-4 and IGFB-6 were expressed by different types of DSC-CM, such as human DPSCs, SHEDs and PDLSCs.

In addition, the difference in the expression and concentration of the factors may be due to the difference in the methods used to collect the conditioned medium. The current study also found that even when the CM is derived from the same type of cells, the expression of the growth factors varied. This result may be explained by the difference in cell numbers, culture medium and condition as well their CM processing method[82].

Apart from various growth factors and cytokines produced by the DSC secretome, this review focused on the effect of the DSC secretome on tissue regeneration. The most abundant evidence was shown in neuroregeneration studies, which was supported by the release of numerous growth factors (NGF, BDNF, NT-3, NTF, GDNF and HGF). These neurotrophins promote the differentiation and growth of neurons and are paramount in treating neurodegenerative diseases as well as neural injuries[51 41 48 43]. Other than neuroregeneration, the DSC secretome has the potential to promote bone regeneration, probably due to the increase in the migration and mineralization potential of the surrounding osteoprogenitor cells by TGF-β1[83]. The TGF-β1-BMP (bone morphogenic protein) signaling pathway has a pivotal role in osseous regeneration through the elevated expression of osteogenic genes in the targeted cells[84,85]. This review also found the potential of DSC secretome in dentine formation for pulp protection, periodontal regeneration and other tissue regeneration, such as cartilage, salivary duct cells, liver and lung tissues, but the

mechanism of action is still inconclusive.

CONCLUSION

In conclusion, evidence showing the effectiveness of the DSC secretome in neuroregeneration and bone regeneration is encouraging. However, data from human studies are still lacking, which could impede the translation of such works into the clinical perspective. Furthermore, the potential for the secretome to be used in the regeneration of other tissue types, such as smooth and skeletal muscle, needs to be explored, as secretome content analysis has shown the presence of the relevant factors.

ABBREVIATIONS

BDNF – brain derived neurotrophic factor
BMP – bone morphogenic protein
CM – conditioned media
CNTF – ciliary neurotrophic factor
DFCs – dental follicle progenitor cells
DPSCs – dental pulp stem cells
DSCs – dental derived stem cells
FGF – fibroblast growth factor
GCSF – granulocyte colony stimulating factor
GDNF – glial cell derived neurotrophic factor
GMSCs – gingival tissues derived mesenchymal stem cells
Hertwig's epithelial root sheath – HERS
HGF – hepatocyte growth factor
IFN-γ - interferon gamma
IGF – insulin like growth factor
IGF-BP – insulin like growth factor binding protein
IL – interleukin
MCP – monocyte chemotactic protein
MSCs – mesenchymal stem cells
NGF – nerve growth factor
NT-3 – neurotrophin-3
PAI – plasminogen activator inhibitor
PDGFR-B – platelet derived growth factor receptor beta
PDLSCs – periodontal ligament stem cells
SCAPs – stem cell from apical papilla
SDF – stromal cell derived factor
SHEDs – stem cells from exfoliated deciduous teeth
TGCs – tooth germ cells
TGF-ß1 – transforming growth factor ß-1
TIMP – tissue inhibitor of metalloproteinase
VEGF – vascular endothelial growth factor

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The authors declare that they have no competing interests.

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