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SYSTEMATIC REVIEW

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Dental stem cells in intraoral regenerative therapy: a systematic review

Células-tronco dentárias na terapia regenerativa intraoral: uma revisão sistemática

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ABSTRACT

Objective: 1) To critically review the published literature on applications of dental stem cells in the regeneration of intraoral tissues. 2) To provide an evidence-based level on research regarding application of dental stem cells in intraoral tissues regeneration. Methodology: This systematic review is conducted as per the JBI guidelines and reported as per the PRISMA. An initial literature search of papers published between 2004 and 2018 yielded 421 manuscripts. Nineteen studies satisfied the inclusion / exclusion criteria and were included for qualitative synthesis. Studies were categorized as animal (11) and human (8) trials. Five independent reviewers critically assessed the included studies. Risk of bias was assessed using SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) bias risk tool, robins-I tool for non-randomised clinical trial and Cochrane Collaboration's Tool for randomised clinical trial. Evidence levels were assessed based on JBI Criteria. Results: Animal trials mainly focused on periodontal regeneration. A high or unclear Risk of bias was more commonly found amongst animal studies. Laboratory, clinical and radiographic evaluation were used to assess the outcome. A total of Eight Human studies were conducted on a total samples size of 153 upon a wide age ranging from seven years to 60 years. Nearly 70% of the human studies used DPSC for regenerating alveolar bone defects. Conclusion: Appropriate well designed double-blind randomized clinical trials of longer duration are yet to be performed. Evidence for the included studies were 1C and 1D as per the JBI Criteria. Stem cell therapy demonstrated promising results in Periodontal tissue and alveolar bone regeneration. However, the number of studies to claim such a benefit are very limited.

KEYWORDS

Dental stem cell (DSCs); Stem cells from human exfoliated deciduous teeth (SHED); Dental pulp stem cells (DPSC); Intraoral application; Human study; Animal study.

RESUMO

Objetivo: 1) Revisar criticamente a literatura publicada sobre aplicações de células-tronco dentárias na regeneração de tecidos intraorais. 2) Fornecer um nível baseado em evidências sobre pesquisas relacionadas à aplicação de células-tronco dentárias na regeneração de tecidos intraorais. Metodologia: Esta revisão sistemática é conduzida de acordo com as diretrizes do JBI e relatada de acordo com o PRISMA. Uma pesquisa bibliográfica inicial de artigos publicados entre 2004 e 2018 resultou em 421 manuscritos. Dezenove estudos satisfizeram os critérios de inclusão / exclusão e foram incluídos para síntese qualitativa. Os estudos foram categorizados como ensaios em animais (11) e humanos (8). Cinco revisores independentes avaliaram criticamente os estudos incluídos. O risco de viés foi avaliado usando a ferramenta de risco de viés do Centro de Revisão Sistemática para Experimentação com Animais de Laboratório (SYRCLE), a ferramenta robins-I para ensaios clínicos não randomizados e a Ferramenta da Colaboração Cochrane para ensaios clínicos randomizados. Os níveis de evidência foram avaliados com base

nos critérios JBI. Resultados: Os ensaios em animais focaram principalmente na regeneração periodontal. Um risco alto ou pouco claro de viés foi mais comumente encontrado entre os estudos com animais. Avaliações laboratorial, clínica e radiográfica foram utilizadas para avaliar o resultado. Um total de oito estudos em humanos foram conduzidos em um tamanho total de amostras de 153 com ampla faixa etária, variando de sete a 60 anos. Quase 70% dos estudos em humanos usaram DPSC para regeneração de defeitos ósseos alveolares. Conclusão: Ensaios clínicos randomizados duplo-cegos apropriados e bem elaborados de maior duração ainda precisam ser realizados. As evidências para os estudos incluídos foram 1C e 1D de acordo com os critérios JBI. A terapia com células-tronco demonstrou resultados promissores na regeneração do tecido periodontal e do osso alveolar. No entanto, o número de estudos para reivindicar tal benefício é muito limitado.

PALAVRAS-CHAVE

Células-tronco dentárias (DSCs); Células-tronco de dentes humanos decíduos esfoliados (SHED); Células-tronco da polpa dentária (DPSC); Aplicação intraoral; Estudo em humanos; Estudo em animais.

INTRODUCTION

Stem cells also known as “progenitor or precursor” cells are defined as clonogenic cells capable of both self-renewal and multi-lineage differentiation which can widely be used in injury to promote repair and tissue regeneration [1]. Many varieties of grownup stem cells are abundantly found in mesenchymal tissues, and these cells are collectively referred to as mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs). Mesenchymal stem cells may be bone marrow-derived, umbilical cord-derived, and fat tissue-derived or dental tissue-derived cells. They are capable of self-renewing by means of dividing and differentiating into more than one tissues inclusive of bone, cartilage, muscle, fats cells, and connective tissue [2]. Tooth stem cells or dental stem cells are stem cells collected from the dental structures, including components of developing tooth, as well as structures within a mature teeth and periodontal ligaments [3]. Dental stem cells are multipotent and are reported to have promising therapeutic approach in reviving structural defects [4].

Presence of stem cells in dental pulp tissues (DPSCs) was initially reported by Yamamura in1985 [5]. However, a breakthrough was achieved in 2000 by Gronthos and his team who identified and isolated odontogenic ancestor population from adult dental pulp, that had the flexibility to regenerate a dentin-pulp-like complex (Gronthos et al. 2002; Karamzadeh and Eslaminejad 2013 [6,7]. In 2003 Dr. Songtao Shi, a Pedodontist discovered dental pulp stem cells by utilizing primary teeth of his daughter & he named them as stem cells from human exfoliated deciduous teeth [8]. Dental tissue-derived mesenchymal stem cells have been documented to be appropriate, because of their easy accessibility, immunosuppressive properties, excessive proliferation ability, and the capability to differentiate into odontoblasts, cementoblasts, osteoblasts, and other different specific mature cells found in dental tissues. Five specific types of dental tissue derived stem cells have been identified which are Dental pulp stem Cells (DPSCs), Periodontal ligament stem cells (PDLSCs), Stem cells from exfoliated deciduous teeth (SHED), Stem cells from apical papilla (SCAP), and Dental follicle progenitor cells (DFPCs). Similar to the most extensively studied bone marrow-derived mesenchymal stem cells (BM-MSCs), these DSCs, derived from oral and maxillofacial areas, have also been shown to possess potent self-renewal/colony forming and multipotent differentiation capabilities [4].

A significant body of literature on different types of DSCs and their applications published in recent years with wide variations in design/methodology is apparent in this literature. An in-depth analysis and comprehension of in vivo research is a prerequisite for evaluating and translating the efficacy of DScs. The focus of this systematic review will therefore be to critically evaluate the applications of different types of dental stem cells specifically for intraoral tissue regeneration. Additionally, we have focussed upon the, the impact of host-related factors (animal model, humans, and defect type) and the scaffold used on the efficiency of dental stem cell and their intraoral applications.

METHODOLOGY

The protocol for the current systematic review is registered with the National Institute of Health Research Database (www.crd.york.

ac.uk/prospero, Protocol I.D. CRD42020206567). This review is conducted in accordance with Joanna Briggs Institute critical appraisal checklist for systematic reviews and reported as per Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines [9].

Literature search and screening strategy

A comprehensive search of scientific papers published between 2004 and 2018 using individual /combination of key words in the following databases IRIS, Scopus, MEDLINE-PubMed, Google Scholar, Cochrane Library and Web of Science was conducted.

Search strategy

Search strategy was based on the following Mesh type keywords:

"(Dental pulp stem cell [MeSH])"; "(dental pulp [MeSH])" AND "(stem cell [MeSH])"; "("dental pulp stem cell" [MeSH]")", Stem Cells from Human Exfoliated Deciduous teeth (SHED)], tissue regeneration, tissue repair, treatment, intraoral application, human study, animal study.

Criteria for considering studies for this review

Population, Intervention, Comparison, and Outcome criteria as mentioned in Table I were employed to improve search strategy. Potentially relevant papers found from the reference lists of related research, review articles and chapters were hand searched. All relevant titles, abstracts published in English over the past 15 years (2004-2018) were identified and retrieved by the first two authors.

Study selection

Initial search yielded 421 abstracts/titles since multiple databases were individually searched, twenty-eight abstracts/titles were identified as duplicates and were excluded. A total of 218 abstracts/titles were excluded because they were literature review/expert opinions (189) and due to language restrictions (29). A total of 175 abstracts/titles qualified for further evaluation. Additionally, 148 articles were excluded after proofreading the abstracts. The reasons for exclusion were as follows: Studies that used stem cells isolated from extra-oral tissues (n= 36) extraoral applications of DSCs (85) and in-vitro studies (27). At this level 27 abstracts/title were considered potentially eligible and sought for further assessment, and full-length articles were retrieved. These full-length articles were independently reviewed by three authors with expertise in the content area so as to establish whether the studies met inclusion criteria. Eight articles were excluded through an unanimous decision of the reviewers. Disagreements amongst reviewers were resolved by discussion and wherever resolution was not possible, a third review author, Associate Professor, Centre for Stem Cell Research & Regenerative Medicine was consulted, and his decision was considered as final. Eventually, a total of 19 articles were sought for qualitative synthesis in this systematic review. The title of the article, name of the journal, and authors and their affiliations were masked and circulated among a panel of five reviewers. The risk of bias of eligible studies was assessed and summarized (Table II, III and IV). Each manuscript was analysed for methodological quality according to a prepared checklist containing 20 items based on ARRIVE [10] and CONSORT [11] guidelines respectively as provided in Table V and VI. Details of the search strategy and reasons for exclusion are documented and presented in a flow chart (Figure 1).

Data extraction

Data extraction was performed manually and data were entered into a structured Microsoft Excel Sheet (Windows Professional plus Version 2016). The characteristics of the study were independently extracted and documented by the same investigators The evidence was compared for accuracy and any differences were addressed and resolved by consensus.

Risk of bias assessment

The risk of bias of eligible studies was assessed using the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) bias risk tool [31],robins-I tool for non-randomised clinical trial [32] and Cochrane Collaboration's Tool for randomised clinical trial [33].

RESULTS

Study characteristics

A total of 11 animal studies and eight human studies were amenable for qualitative synthesis, it

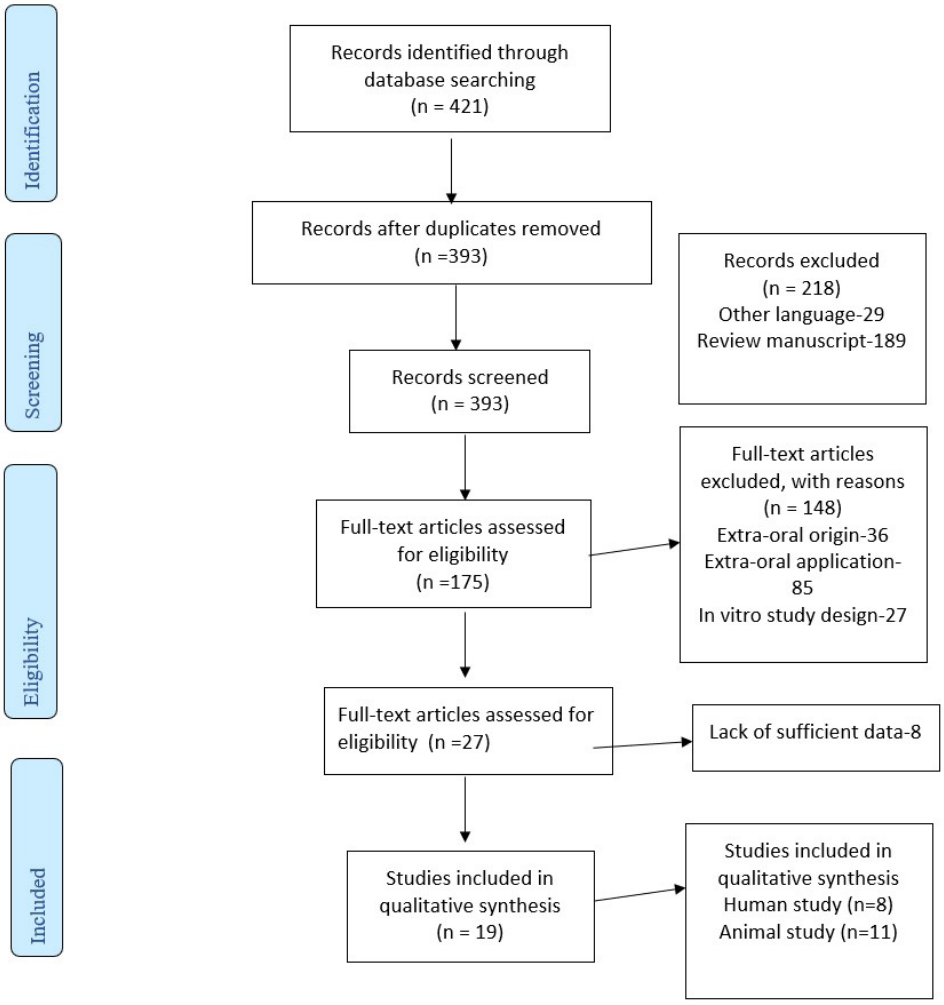

Figure 1 - Flow diagram presenting the results of the literature search and the process used to select studies for the systematic review

Table I - The population, intervention, comparison, and outcome strategy for the structured review question

Research question	Example
The Population	full-text research manuscripts that were written in English describing in vivo analysis of tissue repair and/or regeneration of non-dental tissues using SHED or DPSCs
The Intervention	studies that use DPSCs, SHEDs, SCAPs or DMSCs for dental tissue repair or regeneration.
The comparison	Before and after comparison
The Outcome	Dental tissue regeneration/repair.

Table II - SYRCLE's RoB tool for experimental animal studies

Study	Random sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding (study team)	Random outcome assessment	Blinding (outcome assessor)	Incomplete outcome data	Selective outcome reporting	Other bias
	Selection bias			Performance bias		Detection bias		Attrition bias	Reporting bias	
Moo Seo et al. [12]	U	L	U	U	H	L	H	L	L	U
Sonoyama et al. [13]	U	L	U	U	H	U	H	L	L	U
Liu et al. [14]	L	L	H	H	U	U	U	L	L	L
Zheng et al. [15]	U	U	H	U	H	U	H	H	L	U
Kodonas et al. [16]	U	U	H	H	H	U	H	L	L	U
Nuñez et al. [17]	L	L	U	U	U	L	U	L	L	L
Khorsand et al. [18]	L	L	U	U	U	U	U	H	L	U
Behnia et al. [19]	U	L	U	L	U	U	U	L	L	U
Jahanbin et al. [20]	L	L	U	L	U	L	U	L	L	L
Kuo et al. [21]	U	L	U	U	H	L	H	L	L	L
Tsumanuma et al. [22]	L	L	U	L	U	L	U	H	L	U

L- Low risk of bias; U- Unclear risk of bias; H- High risk f bias

Table III - Risk assessment for non-randomised studies-robins-I tool

Study	Pre-intervention		At intervention	Post-intervention				
	Bias due to confounding	Bias in selection of participants	Bias in classi-fication of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall risk of Bias Judgement
Graziano et al. [24]	Moderate risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk	Moderate risk
Li et al. [27]	Moderate risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk	Moderate risk
Aimetti et al. [28]	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Moderate risk

Table IV - Risk assessment for randomised trials- Cochrane Collaboration's Tool

Study	Random sequence generation	Allocation concealment	Blinding (study team)	Blinding (outcome assessor)	Incomplete outcome data	Selective outcome reporting	Other bias	Overall bias
Aquino et al. [23]	U	U	U	U	L	L	L	U
Chrepa v et al. [25]	U	U	U	U	L	L	U	U
Chen et al. [26]	L	U	L	L	H	L	U	
Ferrarotti et al. [29]	L	L	L	L	L	L	U	L
Xuan et al. [30]	L	L	L	L	L	L	U	L

L- Low risk of bias; U- Unclear risk of bias; H- High risk f bias.

Table V - Qualitative synthesis of included Animal studies

Author and year	Place	Study design	Duration (Months)	Experimental model and sample Immunocompromised	I&E criteria	Origin of stem cell	Source of stem cell	Isolation method	Enzyme for isolation	Scaffold	Defect
Moo Seo et al. [12]	USA	CT	2	Mice-12, rats-6	✓	PDLSC, DPSC	3rd molars,	Enzymatic	C & D	HA/TCP	PDL
Sonoyama et al. [13]	California	CT	6	Minipigs-6	✓	SCAP, PDLSc	3rd molar	Enzymatic	C & D	HA/TCP	Extraction
Liu et al. [14]	China	RCT	3	miniature pigs-14	✓	PDLSC	Cuspids	Enzymatic	C & D	HA/TCP	Periodontitis
Zheng et al. [15]	China	RCT	6	Minipigs-16	✓	SPD	Primary incisor	Enzymatic	C & D	β-TCP	Bone
Kodonas et al. [16]	Greece	CT	2.5	Minipigs-32 implants	✓	S-DPSCs	Incisors, PM	Enzymatic	C & D	Collagen/ PLGA	Bone
Nuñez et al. [17]	Spain	CT	3	Beagle dogs 4	✓	CSCs, PDLCs	1st& 3rd PM	Mechanical	C & D	Collagen	PDL
Khorsand et al. [18]	Iran	RCT	3	mongrel dogs-10	✓	DPSC	Maxillary PM	Enzymatic	C & D	Bio-Oss	PDL
Behnia et al. [19]	Iran	CT	3	Male dogs 4	✓	SHED	EPT	Enzymatic	Trypsin	Collagen	Bone
Jahanbin et al. [20]	Iran	RCT	2	Wistar rats-60	✓	DPSC	Primary molar	Enzymatic	C & D	collagen	Bone
Kuo et al. [21]	Taiwan	CT	2	Lanyu pig-12	✓	hDPSCs	×	×	×	β-TCP/ ACP	Bone
Tsumanuma et al. [22]	Japan	RCT	3	Beagle dogs 8	✓	PDL-MSC	Allogenic, autogenic	Enzymatic	×	b-TCP / collagen	PDL

Table V - Qualitative synthesis of included Animal studies (continued)

Intervention Cases		Control	Primary outcome	Method of evaluation			Attrition	Funding	COI	Loe
Autologous	Heterologous			Laboratory	Clinical	Radiograph				
–	PDLSC+SF	SF	PDL regeneration	RT-PCR, Ab, NBA, WBA	No	No	Nil	IF	No	1c
–	SCAP+SF	Nil	Bio-root formation	HA, IHC, FC, WBA, RT-PCR	No	CT	Nil	IF, UF	No	1c
PDLSC+ SF	–	Nil	PDL regeneration	ICS, FC, SEM, TEM	PLI, SBI, PD, GR, CAL	No	2	IF, UF	No	1c
SPD + SF	–	Nil	Bone regeneration.	IHC, FC, SEM	No	CT	Nil	GF, IF	No	1c
S-DPSCs +SF	–	SF	Bone regeneration.	IHC, HA	No	No	Nil	×	No	1c
CSCs+SF, PDLCs+SF	–	SF	PDL regeneration	IHC, FC, HA	CEJ-GM, CEJ-JE, GM-JE	No	Nil	IF	×	1c
DPSC+SF	–	SF	PDL regeneration	FC, MLD, HME	Suppuration, infection, GR	No	Nil	IF	No	1c
–	SHED+SF	SF	Bone regeneration.	Biopsy, HA, IHC	No	No	Nil	IF	No	1c
–	DPSC+SF	IliacBG	Bone formation	HME, osteoblast activity	No	No	Nil	×	×	1c
–	CSD+ hDPSC α-CSH/ACP+ hDPSC CSD/β-TCP + hDPSC	SF	Bone regeneration.	IVDT, HME	No	WAXD	Nil	×	×	1c
PDLMSC + PGA +SF	PDLMSCs + PGA +SF	PGA+SF	PDL regeneration	FC, DA, APA, ELISA	CEJ, DH	MT	2	IF, UF	No	1c

Table VI - Qualitative synthesis of included Human studies

Author	Place	Study design	Duration (months)	Cases			Control			I&E criteria	Origin of stem cell	Isolation method	Enzyme for isolation	Scaffold	Defect
				N	M: F	Mean age(yrs)	N	M: F	Mean age						
Aquino et al. [23]	Italy	RCT	12	7	1:6	×	7	1:6	×	✓	DPSCs	Enzymatic	α-MEM	Collagen	Bone
Graziano et al. [24]	Italy	CT	9	1	1:0	42	_	_	_	✓	DPSCs	Mechanical	PS	Collagen	Bone
Chrepa et al. [25]	USA	RCT	-	20	8:12	44	×	×	×	✓	AMSCs	Mechanical	BM	Absent	Periapical
Chen et al. [26]	China	TB RCT	12	20	2:18	26.1±4.4	24	6:15	30±7.9	✓	PDLSCs	Enzymatic	Trypsin	Absent	Bone
Li et al. [27]	China	CT	9	2	0:2	34	_	_	_	✓	DPSCs	Enzymatic	C & D	β-TCP	PDL
Aimetti et al. [28]	Italy	CT	12	11	6:5	51.2±6.1	_	_	_	✓	DPSCs	Mechanical	×	Collagen	Bone
Ferrarotti et al. [29]	Italy	DB RCT	12	15	8:7	51.9±8.4	14	6:8	49.4±9.3	✓	DPM	Mechanical	×	Collagen	PDL
Xuan et al. [30]	China	TB RCT	24	30	26:4	7.1±0.9	10	7:3	7.1±0.7	✓	DPSCs	Enzymatic	Trypsin	Absent	Pulp

Table VI - Qualitative synthesis of included Human studies (continued)

Intervention		Primary outcome	Method of evaluation			Attri-tion	Funding	COI	LOE
Cases	Control		Lab	Clinical	Radiograph				
DPSC+SF	×	Bone regeneration	BS, IF	PD, CAL	IOPA	Nil	GF	No	1c
DPSC+SF	NA	Bone regeneration	BS	No	CT	Nil	UF	×	1c
MSCs into RC	×	Influx of Mscs	IHC, FC	No	IOPA	Nil	UF, IF	No	1d
Bio-oss+cellsheet	Bio-oss	Bone regeneration	IHC	CAL, GR, PD	IOPA	10	UF	No	1c
DPSC+SF	NA	PDL regeneration	No	PI, BI, PD, CAL, GR	IOPA	Nil	UF	×	1c
DPSC+SF	NA	Bone regeneration	No	PD, CAL, GR	IOPA	Nil	IF	No	1c
MIST + DPM	MIST+scaffold	PDL regeneration	IF	CAL gain, PD reduction	IOPA	1	SF, IF	No	1c
hDPSC	Apexification	Pulp regeneration	CWD, LDF, ELISA	No	RVG, CBCT	4	IF	No	1c

(×)- Not mentioned; (-)- Not reported.

was performed separately for animal and human studies using their respective guidelines.

A total of 11 studies upon animal models were conducted over a period 13 years, from Iran (3) followed by China (2). Five studies were reported to be Randomized clinical trial and 6 studies were clinical trial. These studies were performed on mini pigs (5), dogs (4) and rat/mice (2). Inclusion and exclusion criteria were clearly mentioned in all the studies. Most of the studies utilized DPSC (5) and PDLSC (4). Five studies utilized autologous stem cells (cells are derived from the same individual) and 5 studies used heterologous stem cell (cells from a mixed population of donor cells). One of the studies was done with both autologous and heterologous stem cell. Majority of the studies utilized enzymatic (9) technique for isolation of tissue followed by mechanical method (1) using either collagen and dispase (8) or trypsin (1). Collagen scaffold was used among most of the studies (5) followed by HA/TCP (3) & β-TCP (3). Other scaffold used were PLGA (1), ACP (1) and Bio-oss (1). The experimental model underwent stem cell implantation for periodontal defect (4), bone defect [mandible defect (3), extraction socket defect (2), maxillary defect (1)] and periodontitis (1) with or without scaffold. Three studies did not provide any intervention for control group. The primary outcome of most of the studies was bone regeneration (5) and PDL regeneration (5). One study has focused on bio root formation. The outcome was assessed through laboratory, clinical and radiographic evaluation. Laboratory evaluation included specific tests suchas In vitro dissolution test(IVDT), Histomorphometry evaluation(HME), Flow cytometry(FC),Immunohistochemistry(IHC),Histology analysis(HA), Differentiation assay(DA), Alkaline phosphatase activity(APA), ELISA, Scanning electron microscopy(SEM), Immunocytochemistry(ICS), Transfection of eGFP gene(TEM), Northern blot analysis(NBA),

Western blot analysis(WBA), Reverse transcriptase polymerised chain reaction(RT-PCR) and Antibodies(Ab). Clinical assessment focussed primarily on aspects of periodontology. Clinical evaluation was assessed by plaque index(PI), Sulcus bleeding index(SBI), Probing depth(PD), Gingival recession(GR), Clinical attachment loss(CAL),Distance from Cementoenamel junction to gingival margin(CEJ-GM), Cementoenamel junction to junctional epithelium(CEJ-JE), Gingival margin to junctional epithelium(GM-JE), Suppuration, infection, Gingival recession and cementoenamel junction (CEJ).Radiographic evaluation was performed using Computed tomography (CT), Microcomputed tomography (MT) and Wide-angle X-ray diffraction (WAXD). Attrition was reported to be less than 15%, three authors have not spotted COI.

Analysis of human studies over a period of 9 years 2004-2018 published from across the globe, mostly from Italy (4) and China (3) are conducted upon a population of 153 subjects with an age ranging from 7 years to 60 years. Majority of the studies were non-blinded clinical trials (63%) conducted over a period of 9 to 24 months. Minor variations between cases and controls with regards to age and notably wider variations in gender distributions (female predominance) were apparent. Inclusion and exclusion criteria have been clearly mentioned in all the studies. Most of the studies have utilized DPSCs (5), isolated using the enzymatic (4) or mechanical (4) technique and stored either in trypsin (2), alpha essential medium (1), collagenase dispase (1), physiologic solution (1) or basal medium (1). Collagen scaffold was used among most of the studies (4) followed by no scaffold studies (3) and β-TCP (1). The defect that were attempted to correct using stem cells were alveolar bone defect (4) and PDL defect (2), periapical lesion (1) and pulp necrosis (1) with or without scaffold. Conventional treatment prior to the stem cell therapy was reported to be performed in most of the studies, but the details thereof have not been reported. primary outcome of most of the studies was bone regeneration (4). Others have focused on PDL regeneration (2), pulp regeneration (1) and influx of MSCs (1). outcome was assessed through laboratory, clinical and radiographic evaluation. Laboratory evaluation included specific tests such as bone sampling (BS), Immunohistochemistry (IHC), Flow cytometry (FC), Immunofluorescence (IF), ELISA, Continuous wave Doppler (CWD) and Lesser Doppler flowmetry (LDF). Clinical evaluation was assessed through measuring pocket depth (PD), clinical attachment level (CAL), gingival recession (GR), plaque index (PI) and Bleeding index (BI). Routine IOPAs, CBCT, RVG and CT were used to assess the mineralisation, bone level, lamellar bone formation, root length and apical foramen width in various studies. Attrition was reported to be less than 30%.

Risk of bias assessment

Assessment of Risk of bias for animal studies revealed either unclear or high degree of bias across all domains. All studies reported similar experimental and control groups at baseline, minimizing selection bias. Although the assignment of subjects to the experimental and control groups was claimed to be random, random sequence generation was not reported in any of the studies. Hence it was judged as “unclear”. The domains of allocation concealment, randomly housing, blinding of investigators, caregivers, random outcome assessment and blinding of outcome assessor were found to be either “unclear” or having “high risk of bias”. Reporting bias was assessed using signalling questions and were found having a “low risk of bias”. The details of the assessment are mentioned in Table II.

Robins-I tool (Table III) assessment for non-randomised trials, showed the presence of “moderate risk of bias” in almost all the included studies. Within the domains, subgroup analysis revealed “moderate risk of bias” in controlling confounding factors. Post-intervention analysis demonstrated a “low risk of bias” in “deviations from intended interventions”, “missing data”, “measurement of outcomes” and “selection of the reported result”.

Risk of bias assessment for randomized control trial conducted upon humans (Table IV) showed that most of the studies were having an “Unclear” to “low risk of bias”. “unclear risk of bias” was seen in “allocation concealment” and “other sources of bias”. “Random sequence generation” and “Selective reporting” demonstrated a “low risk of bias”.

DISCUSSION

A Russian histologist named Alexander Maksimov, in 1908, reported the existence

of the somatic cell as a part of his theory of haematopoiesis [34]. Stem cells are fascinating for scientists because of their ability to give rise to an extremely specialised cell kind or organism and their almost endless self-renewal potential. Research on stem cells is advancing our knowledge of how a full organism develops from one cell and the way broken cells get replaced by healthy cells in adult organisms [35].

This growing field of research is also leading scientists to explore the potential for cell-based therapies to treat diseases. Stem cell research and therapy present new hope as an exciting therapeutic option for patients and good future prospect for scientists [36]. Stem cells additionally referred to as "progenitor or precursor" cells are biological cells found in all cellular organisms that can divide (through mitosis) and differentiate into numerous specialised cell sorts and can self-renew to produce additional stem cells [37].

Biologically, mesenchymal cells are primarily liable for the formation of nearly all dental, oral, and craniofacial structures. Mesenchymal stem cells in the adult, are demonstrated, in tissue engineering, to get key dental, oral, and craniofacial structures [38]. Most dental and craniofacial structures are promptly available, thereby providing a convenient platform for biologists, bioengineers, and clinicians to track tissue-engineered prototypes. Human stem cells were derived from the dental pulp, exfoliated deciduous teeth, the periodontal ligament, the dental follicle and the dental papilla. Advances in this field have led to significant progress in tissue repair and regeneration processes in dental tissue. Dentistry has found its way in restoring missing teeth and its contiguous tissues to restore the function through the delivery of the stem cells, bioactive molecules, or synthetic tissue engineered in the laboratory.

Contemporary dental practice is largely based on conventional, non-cell-based therapies that rely on durable materials from outside the patient's body. The mainstream options for the reconstruction of dental, oral, and craniofacial structures have been amalgam, composites, metal implants, synthetic materials, and tissue grafts from human cadavers and other animals [38]. Despite varying degrees of clinical effectiveness, conventional materials have inherent drawbacks, such as possible immune rejection, donor transmission of pathogens, and the general inability of conventional materials to remodel recipient tissues and organs [30]. With advancing research in biotechnology, regenerative capability of our own living body tissue has opened a new era of tissue engineering.

Evidence of DSCs in the regeneration of various tissues and organs, such as bones, vascular system, liver, pancreas, cornea and neurodegenerative diseases among animals of other species, such as pigs, rats and mice is reported by Daltoé et al. [39] in 2014. Overview of 14 in vivo studies using human tissue as the origin of the cells concluded that the use of DPSCs and SHED appears to be successful for these applications. However, very few studies were found regarding the true potential of these cells to promote functional recovery of neuronal tissue, blood vessels, muscle, cartilage, or other tissues.

In 2018, Gaubys et al. [40] quantified the effect size of stem cell therapy on the regeneration of periodontal tissue complex in animal models in a meta-analysis of 10 RCTs and concluded that Such therapy has most significance on cementum regeneration meanwhile alveolar bone regeneration is influenced by the least amount. The applicability of stem cells from bone marrow derived stem cells, adipose derived stem cells, stem cell from iliac bone, umbilical cord mesenchymal stem cells and DSCs have been reported for the treatment of periodontitis, maxillofacial bone regeneration, pulp regeneration after necrosis, as well as the development of new teeth to varying levels of success.

In 2017, Kanwal et al [41] conducted a systematic review on the current status of stem cell regeneration in intra-oral applications by reviewing 156 animal studies and 16 human studies. They concluded that ongoing research and development of new scaffolds, understanding different signalling molecules and their indications, understanding gene expression and proteomics of stem cells are the future directions that will help us to achieve successful regeneration.

In a qualitative synthesis of 56 articles by Leyendecker et al. [42] in 2018 revealed that hDPSCs and SHED can be applied for bone tissue regeneration upon animals and humans. The findings of this systematic review open new doors for bone tissue engineering in patients with bone defects.

To the best of our knowledge, systematic reviews specifically on the applicability of dental stem cells for the regeneration of orofacial tissues are not reported. There is thus a need to critically evaluate and assess the evidence of the applicability of DSC in orofacial structures.

The objective of this systematic evaluation, based on 19 research reported over a period of 14 year, is to comprehend the intraoral applications of the various types of dental stem cells and to systematically evaluate the current status of dental stem cell regeneration in multiple hard and soft intraoral tissues.

Included 11 animal studies were critically evaluated using a checklist of 19 different parameters and 8 human studies in 18 categories. It has been reported that the duration of animal studies included ranged from two to six months, while human studies ranged from nine months to 24 months. Most of the animal studies used DPSC to replace a periodontal defect whereas the main focus of human research was on bone regeneration through DPSC.

Comprehensive laboratory investigation is reported amongst animal research whereas a more detailed clinical assessment has been reported among human studies. Analysis revealed the use of heterologous stem cell amongst animal research necessitating detailed preclinical investigation. Homologous stem cells have been used amongst human studies and the main focus was on the outcome.

Appropriate well designed double-blind randomized clinical trials are yet to be performed to confirm the true regenerative power of dental stem cells. Several primary parameters should be optimised through more clinical study, including the necessary stem cell density and availability, as well as suitable strategies for their use (either alone or in combination with scaffolds, site injection, etc.). The other major issue regarding the clinical use of oral stem cells is the availability of the cells over time. For example, dental pulps stem cells or exfoliated deciduous tooth stem cells are not available throughout a patient's lifetime [43]. Since this availability is essentially greater at an early age, oral stem cell banking by cryopreserving these oral stem cells for possible clinical use can be a potential solution. However, such a possibility is not only time-consuming and costly but limits the use of some oral stem cells as a clinical strategy. The use of oral stem cells for dental tissue regeneration is nonetheless an important biotechnological innovation with the potential of providing significant benefits to overcome the effects of dental diseases.

The stem cell research pertaining to orofacial region is meagre. It should be realised that the need for dental treatment is always considered to be of less priority. Consequently, stem cell research has traditionally focused upon cardiovascular diseases, neurological problems, leukaemia, etc. which are more serious and life-threatening [44].

This qualitative synthesis further proves that dental stem cells are effective in regenerating animal and human dental defects like bone defect and periodontal defect. Results of this review indicate that research on dental stem cell-based therapy with improvised scientific methodology need to be conducted upon the applicability of DSCs in the regeneration of dental tissues. These findings are compatible with the previous reported systematic reviews [39-42].

Risk of bias assessment revealed a high degree of bias amongst animal studies and unclear risk of bias in most of the human studies.

In the light of current research trends and advances, it is now appropriate to consider switching from preclinical animal studies to human studies with sufficient sample size and more robust scientific methodology before cell therapy can be instituted as a part of routine dental practice. we believe that this is the best possible review of evidence on the subject with an evidence level of 1c for animal studies and 1c and 1d for human studies.

CONCLUSION

We have entered a new phase of orofacial tissue regeneration, where stem-cell-based therapies can be used to strengthen and increase clinical outcomes. Current active research fields of stem-cell therapy in dentistry concentrates on tissue engineering and chair-side cellular grafting strategies that could result in more reliable regenerative outcomes for the future. Despite abundance in the literature, this systematic review draws attention to the fact that there is still an acute short of high-quality data on the application of DSCs in intraoral tissue regeneration. In this era of evidence-based practice there is a need to carry out well designed studies and to present the results and report outcomes in a more scientific methodology.

Author Contributions

Study conception and design: APK, NF; Data collection: APK; Analysis and interpretation of results: APK, NF, MKB; Draft manuscript preparation: APK, NF, MKB, HB, JP. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Regulatory Statement

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ABBREVIATION

DSCs- dental stem cells

CT-Clinical trial

RCT-Randomised clinical trial

DB-RCT- Double blinded Randomised clinical trial

TB-RCT- Triple blinded Randomised clinical trial

PDLSCs- Periodontal ligament stem cells

DPSC-Dental pulp stem cell

SCAP- Stem cell from apical papilla

SPD-stem cell from pig deciduous teeth

CSCs-cementum derived cells

DPM-dental pulp micrograft

SHED-stem cells from human exfoliated deciduous teeth

MSCs- Mesenchymal stem cells

C & D- Collagen & Dispase

HA/TCP- Hydroxyapatite/ Tricalcium Phosphate

PLGA-poly lactic co-glycolic acid

MEM-Minimal essential medium

PS-Physiologic solution

BM-Basal media

PDL-Periodontal ligament

SF-Scaffold

MIST-minimally invasive surgical technique

CSD-Calcium sulphate dihydrate

CSH-Calcium sulphate hemihydrate

ACP-amorphous calcium phosphate

BG-Bone graft

RT-PCR- Reverse transcriptase polymerised chain reaction

Ab-Antibody

NBA-Northern blot assay

WBA-Western blot assay

HA-Histologic analysis

IHC-Immunohistochemistry

FC-Flowcytometry

ICS-Immunocytochemistry

SEM-Scanning electron microscope

TEM-Transfection of EGEF gene

MLD-multilineage differentiation

HME-Histomorphometry evaluation

IVDT-In vitro dissolution test

DA-Differentiation assay

APA-Alkaline phosphatase activity

ELISA-enzyme linked immune sorbent assay

PLI-Plaque index

SBI-Sulcus bleeding index

PD-Probing depth

GR-Gingival recession

CAL-Clinical attachment loss

CEJ-Cementoenamel junction

GM-Gingival margin

JE-Junctional epithelium

DH-Dentin height

BI-Bleeding index

BS-Bone sampling

CWD-Continuous wave doppler

LDF-Laser doppler flowcytometry

CT-Computed tomography

WAXD-Wide angle X-ray diffraction

MT-Microcomputed tomography

RVG-radio visiography

CBCT-Cone beam computed tomography

IOPA-intraoral periapical

IF-Institutional funding

UF-University funding

GF-Government funding

SF-Self funding

✗-Not mentioned

(-)- Not reported