



Review

# Dental Stem Cell-Based Therapy for Glycemic Control and the Scope of Clinical Translation: A Systematic Review and Meta-Analysis

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**Abstract:** Background: The tooth is a repository of stem cells, garnering interest in recent years for its therapeutic potential. The aim of this systematic review and meta-analysis was to test the hypothesis that dental stem cell administration can reduce blood glucose and ameliorate polyneuropathy in diabetes mellitus. The scope of clinical translation was also assessed. Methods: PubMed, Cochrane, Ovid, Web of Science, and Scopus databases were searched for animal studies that were published in or before July 2023. A search was conducted in OpenGrey for unpublished manuscripts. Subgroup analyses were performed to identify potential sources of heterogeneity among studies. The risk for publication bias was assessed by funnel plot, regression, and rank correlation tests. Internal validity, external validity, and translation potential were determined using the SYRCLE (Systematic Review Center for Laboratory Animal Experimentation) risk of bias tool and comparative analysis. Results: Out of 5031 initial records identified, 17 animal studies were included in the review. There was a significant decrease in blood glucose in diabetes-induced animals following DSC administration compared to that observed with saline or vehicle (SMD:  $-3.905$ ; 95% CI:  $-5.633$  to  $-2.177$ ;  $p = 0.0004$ ). The improvement in sensory nerve conduction velocity (SMD:  $4.4952$ ; 95% CI:  $0.5959$  to  $8.3945$ ;  $p = 0.035$ ) and capillary-muscle ratio (SMD:  $2.4027$ ; 95% CI:  $0.8923$  to  $3.9132$ ;  $p = 0.0095$ ) was significant. However, motor nerve conduction velocity (SMD:  $3.1001$ ; 95% CI:  $-1.4558$  to  $7.6559$ ;  $p = 0.119$ ) and intra-epidermal nerve fiber ratio (SMD:  $1.8802$ ; 95% CI:  $-0.4809$  to  $4.2413$ ;  $p = 0.0915$ ) did not increase significantly. Regression ( $p < 0.0001$ ) and rank correlation ( $p = 0.0018$ ) tests indicated the presence of funnel plot asymmetry. Due to disparate number of studies in subgroups, the analyses could not reliably explain the sources of heterogeneity. Interpretation: The direction of the data indicates that DSCs can provide good glycemic control in diabetic animals. However, methodological and reporting quality of preclinical studies, heterogeneity, risk of publication bias, and species differences may hamper translation to humans. Appropriate dose, mode of administration, and preparation must be ascertained for safe and effective use in humans. Longer-duration studies that reflect disease complexity and help predict treatment outcomes in clinical settings are warranted. This review is registered in PROSPERO (number CRD42023423423).

**Keywords:** dental stem cells; mesenchymal stem cells; dental pulp; exfoliated teeth; gingiva; periodontal ligament; diabetes mellitus; diabetic poly neuropathy; translation



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## 1. Introduction

In 1957, when Thomas et al. published their seminal report on allogeneic hematopoietic stem cell (HSC) transplants in humans [1], they opened the door to a revolutionary therapy for leukemia. Since then, stem cell (SC) research in diseases such as retinopathy, dementia, and diabetes mellitus has been advancing by leaps and bounds [2].

It wasn't until the 1960s, when Friedenstein et al. identified a group of cells providing a scaffold for HSCs within bone marrow, that mesenchymal stem cells came to light [3]. These stromal cells, termed as mesenchymal stem cells (MSCs) by Caplan in 1991, are plastic, possessing the ability to differentiate into various mesenchymal cell lines and regenerate injured tissues [4]. They migrate to sites of injury and stimulate the proliferation and differentiation of native progenitor cells [5]. MSCs have relatively low immunogenicity [5,6]. Human leucocyte antigen (HLA) molecules are a group of antigen-presenting proteins which are responsible for initiating allogeneic graft rejection [7]. MSC transplants express low levels of HLA class I molecules, do not express HLA class II molecules [7], and remain inconspicuous to cytotoxic T and natural killer (NK) cells [5,8]. In addition, they stimulate the production of regulatory T and B cells [9], a group of specialized lymphocytes that suppress immune response, thereby maintaining homeostasis and immunological tolerance [10].

It is estimated that by 2030, diabetes mellitus will be prevalent in about 643 million adults worldwide [11]. Aside from diseases such as ischemic heart conditions and COVID-19, it was a leading cause of death in 2021, and the global cost for diabetes-related health care was at least USD 966 billion [11]. Uncontrolled diabetes is of grave concern, especially in the post pandemic era, as it complicates recovery and affects the prognosis of almost every infection and disease. Type 1 diabetes (T1DM) is caused by autoimmune destruction of islets of Langerhans, resulting in endogenous insulin deficiency [12]. Its pathogenesis is influenced by genetic as well as environmental factors and is most commonly diagnosed in adolescents and young adults [12]. Type 2 diabetes (T2DM) is characterized by insulin resistance and the inability of islet cells to produce sufficient insulin to compensate, leading to relative insulin deficiency [12]. It is most commonly diagnosed in middle aged adults and is often associated with obesity [12].

Approximately 50% of diabetic adults are eventually afflicted with polyneuropathy (DPN) [11]. It is characterized by axonal degeneration [13,14] caused by pro-inflammatory cytokine-releasing metabolic cascades, which result in structural and functional changes in endoneurial and microvascular tissues [15,16]. DPN is associated with a high risk for foot ulcers and lower limb amputations [16]. Treatment involves regular screening, stringent glycemic control, and pain alleviation [16].

Islet cell transplantation is an alternative therapy for diabetes mellitus, but is associated with the risk of graft rejection and paucity of donor sources [14]. Bone marrow mesenchymal stem cells (BMMSCs) are capable of differentiating into insulin secreting cells [8]. However, retrieval is often accompanied by pain [8], nerve injury [17], and low cell yield upon harvest [8]. Hence, the umbilical cord, placenta, and teeth, which are discarded after removal, have gained ground as alternate MSC reserves [8,17].

The primitive tooth organ and supporting structures are formed by complex interactions of the neural crest with epithelial and mesodermal components [18]. The dental follicle and papilla formed in turn give rise to gingiva, periodontal ligament, and dental pulp [8]. Hence, these ectomesenchymal tissues retain the stemness of neural crest cells [8,18]. There are six types of stem cells of dental origin (DSCs) that have been isolated and characterized so far: dental pulp stem cells (DPSCs), stem cells from the pulps of human exfoliated deciduous teeth (SHED), dental follicle precursor cells (DFPCs), stem cells from apical papilla (SCAP), periodontal ligament stem cells (PDLSCs), and gingival mesenchymal stem cells (GMSCs) [8,9,18]. SCs harvested from these tissues are able to transdifferentiate into neuronal-like and pancreatic  $\beta$  cells [8,9,17], and this ability can be traced back to the neural crest and to similarities between neuronal and pancreatic  $\beta$  cells [8].

Following extraction, exfoliation, or gingival surgery, ideally under sterile conditions and minimal trauma, the tooth or periodontal tissue is preserved to maintain the viability of stem cells until their retrieval [19]. After the surface of the tooth is cleansed, the tissue is retrieved from the tooth, minced, and digested in collagenase [19]. The cells undergo neutralization and centrifugation [19]. They are then trypsinized and can differentiate

into insulin releasing islet-like cells after undergoing several passages in culture [19]. The stem cell activity in a tooth varies, and is contingent on factors such as donor age, tooth morphology, DSC type, tissue health, and conditions during retrieval [20]. For instance, in one study, six lines of SCs were obtained from the pulps of six deciduous teeth of children aged 4–8 years, whereas two DPSC lines were obtained from six permanent teeth of donors aged 55–67 years [20].

DSCs have more robust population doubling rates than do BMMSCs [8]. They are also able to differentiate into odontogenic, osteogenic, chondrogenic, adipogenic, and epithelial cell lineages under specific conditions, thus possessing the potential for application in the regeneration and repair of tissues that arise from all three germ layers [19,21]. There is ongoing research for their use in pulp and dentin regeneration, osteoporosis, rheumatoid arthritis, and liver fibrosis, among other diseases [8,18,20,21].

DSCs can serve as replenishable sources of islet cells; hence, they hold allure in diabetology. Studies have been done to elucidate their effects; however, to our knowledge, a study which systematically reviews and quantifies existing data on the use of various DSCs in diabetes mellitus has not yet been undertaken. Hence, we conducted a systematic review and meta-analysis to appraise the extent of evidence in relation to the focused question: “Do dental stem cells reduce blood glucose and alleviate polyneuropathy in diabetic animals compared to animals administered with saline or vehicle?” In addition to testing this hypothesis, we discern the scope of translation of this novel therapy to humans.

## 2. Methods

This systematic review was reported in accordance with the guidelines outlined in the PRISMA 2020 statement [22], an updated version of Preferred Reporting Items for Systematic Reviews and Meta-Analysis, which is a set of guidelines used to improve the transparency and quality of systematic reviews (PRISMA 2020 checklists are available as Supplementary Tables S1 and S2). The review is registered in PROSPERO, an international database of systematic reviews (registration number CRD42023423423).

### 2.1. Search Strategy

The search was defined to identify studies that evaluated the effects of stem cells of dental origin (DSCs) on blood glucose or DPN parameters, when administered in diabetic animals. The search was conducted in multiple stages. In the first step, a search was performed using the Cochrane Library (Issue 12, 2015), PubMed (MEDLINE—1996), Scopus (1990), Ovid (Embase-1974), and Web of Science (1996) electronic databases. A search was also carried out for grey literature in OpenGrey (openSIGLE 2007). There were no restrictions regarding publication date, and the last search was performed in July 2023. The titles and abstracts were read to determine if they potentially fit the inclusion criteria. Duplicate articles were removed. If the full text was not available from the databases for review or additional information was deemed necessary for selection, the corresponding authors of potential studies were contacted via email. Following this, the full text of potential studies was read to evaluate if they should be included in the review. In the next stage, during July 2023, reference lists of all articles included in the initial step were manually searched. There were no language restrictions in our search strategy.

The search was conducted using MeSH (medical subject heading) terms belonging to the categories of disease, intervention, and population in different permutations and combinations—“Diabetes” [MeSH] OR “Diabetes Mellitus” [MeSH] OR “glucose” [MeSH] OR “insulin” [MeSH]) AND “dental stem cells” [MeSH] OR “Periodontal stem cells” [MeSH] OR “gingival stem cells” [MeSH] OR “glycemic control” [MeSH]. Free text was also used, which included “diabetes”, “diabetes mellitus”, “diabetes mellitus type 1”, “diabetes mellitus type 2”, “glucose”, “insulin”, and “dental stem cells”, “periodontal stem cells”, “gingival stem cells”, and “glycemic control”. In addition, search filters, as described by Hooijmans et al. (2010) [23], were employed for a more comprehensive retrieval of animal

studies from the databases. The full version of the search filters used is presented in Supplementary Table S3.

### 2.2. Selection Process

The titles and abstracts of all records were screened by all authors (P.T., V.T., S.J., and G.Y.) independently. In all cases, disagreements among the reviewers regarding which articles to read through full text were resolved through discussions. All authors then read through full text articles to determine inclusion. The reference lists of selected articles were read by the authors independently. The final selection of studies was discussed, and in case of disagreements regarding inclusion of articles, a consensus was reached among the authors. Automation tools were not used at any stage of the defined search protocol.

### 2.3. Inclusion Criteria

1. Animal studies, regardless of species, age, and gender, published in or before July 2023, were included.
2. Studies in which the disease model was induced diabetes mellitus (Type 1 or Type 2), with any manner of disease induction, were included.
3. Studies which included control groups with animals administered with saline or vehicle for comparison were included in the review.
4. Studies which used dental stem cells, i.e., stem cells of dental origin, as the intervention were included, regardless of dose, timing, frequency, preparation, and route of administration. There were no restrictions regarding the source or portion of the tooth or its supporting tissues from which the DSCs were isolated.
5. Studies using blood glucose and/or verifiable parameters of diabetic polyneuropathy, such as sensory and motor nerve conduction velocity (SNCV and MNCV), as outcome variables were included.
6. Grey literature, such as preprints, dissertations, theses, unpublished manuscripts, and conference papers was also reviewed to determine if it met the inclusion criteria mentioned above.

There were no restrictions in regards to the language of the articles.

### 2.4. Exclusion Criteria

1. Animal studies which did not include diabetic models were not included in the review. In addition, animal studies which did not include diabetic controls administered with saline or vehicle for comparison with diabetic animals administered with DSCs were excluded.
2. Studies in which diabetes mellitus was induced after DSC administration were excluded.
3. Studies which did not use stem cells of dental origin were not included in the review.
4. Studies which did not measure blood glucose and/or DPN parameters, or in which these variables were not measured using valid methods, were not included in the review.
5. In vitro studies, surveys, and questionnaires were not included.
6. Reviews and duplicate articles were excluded.

The complete list of studies that were excluded after reading the full text is available upon reasonable request from the corresponding author.

### 2.5. Data Extraction

The data extracted from selected studies regarding the study design included country of origin, method of conducting the experiment, and study duration. Data regarding the animal model included species, age of animals at the start of the experiment, method of diabetes induction, and time of sacrifice. Information regarding the type of DSCs used in the study, source, preparation, dose, and route of administration was extracted. Changes in primary outcome variables, i.e., fasting and/or random blood glucose, and DPN parameter values, such as SNCV and MNCV, from baseline to the end of the experiment following DSC administration, were included. Other outcome measures were also noted, if present.

All authors were involved in data extraction. In case of unreported or missing data, the authors of the selected studies were contacted via email. WebPlotDigitizer, a web-based tool (<https://automeris.io/WebPlotDigitizer> (accessed on 3 August 2023)) was used if data was presented graphically.

#### 2.6. Assessment of Internal Bias in Articles

The internal bias in each study was assessed using the SYRCLE (Systematic Review Center for Laboratory Animal Experimentation) tool by Hooijmans et al. (2014) [24], which in turn is based on Cochrane's RoB (risk of bias) tool [25]. The assessment of internal bias was conducted by each of the authors (P.T., V.T., S.J., and G.Y.) individually.

#### 2.7. Assessment of External Validity

The external validity of the studies was analyzed using a table that examined data from included studies to determine the scope of translation of DSC therapy to humans.

#### 2.8. Meta-Analysis

Review Manager (RevMan), version 5.4 (The Cochrane Collaboration, 2020) [26], and MAJOR with jamovi statistical software (version 2.3) [27] were used to perform quantitative analyses when more than two studies provided data for each outcome of interest.

Due to anticipated differences in design and effect sizes between studies, random effects model was used for calculating summary effect estimates [28]. The inverse variance method was used to combine results across studies because with this method, more weight is assigned to studies with smaller standard errors; hence, there is greater precision in the overall summary effect [28]. Standardized mean difference (SMD) was used as the outcome measure due to inter study differences in units and methods for measuring outcomes [29]. Hedges' adjusted g method [30] was used to compute SMD, as it adjusts for small sample bias. The SMD was presented with 95% confidence interval (CI), which represented the range in which one can be 95% certain that the true value of the SMD lies [31]. A *p*-value of less than 0.05 was considered statistically significant for the pooled summary effects.

DerSimonian–Laird estimator was used to calculate the extent of heterogeneity (i.e.,  $\tau^2$ ) among the studies [32]. Knapp–Hartung method [33] was used to reduce the risk of Type 1 error (i.e., incorrect rejection of a null hypothesis that is actually true), particularly in analyses that constituted a small number of studies. In addition, Cochran's Q-test and its *p* value were calculated to determine potential heterogeneity [34]. A *p*-value of less than 0.05 was indicative of presence of heterogeneity, which cannot be attributed only to chance [28].  $I^2$  statistic was used to examine the degree of heterogeneity [28].  $I^2$  values of 50% or more were deemed as indicative of substantial heterogeneity [28]. Further, the prediction interval, which helps demonstrate the range of true effects that may be expected in future similar studies [35,36], was also calculated for each outcome.

Studentized residuals were calculated to identify potential outliers, i.e., studies with 95% confidence intervals that were outside those of the pooled effect [37]. The equation  $100 \times (1 - 0.05/(2 \times k))$  th percentile of a standard normal distribution, i.e., Bonferroni correction [38] with 2 sided  $\alpha = 0.05$  for *k* number of studies, was calculated for each analysis, and if the studentized residual range of a study exceeded this value, it was considered an outlier in the model. Studies that may have excessively leveraged the pooled effect were identified using Cook's distances [39]. If the Cook's distance of a study was larger than the median plus six times the interquartile range of the Cook's distance, it was considered to be influential in the context of the analysis [39].

Multiple reports of the same study describing different outcome measures were identified during data collection. In such cases, data from all reports was collected and presented as a single study in the systematic review, and relevant quantitative data from only one report per study was used in each meta analysis to avoid unit of analysis errors [40]. For multiple-arm studies, all relevant intervention groups were combined to create one group for a single paired comparison with the control group to avoid 'double-counting' of an-

imals [41]. For studies with a cross-over design, each paired analysis was estimated by calculating mean difference and standard error of the mean difference and entering the data in the analysis in the form of generic inverse variance outcome [41].

### 2.8.1. Subgroup Analysis

Studies included in the meta-analysis for DSC effect on blood glucose were split into subgroups to examine potential sources of heterogeneity among studies [42] and to measure various treatment effect estimates. Species differences and DSC type were used as variables to check for possible interactions among subgroups, since it was anticipated that these aspects may be potential sources of heterogeneity.

A test for significance which determines heterogeneity across subgroups was conducted, as described by Borenstein et al. (2009) [43]. The  $I^2$  statistic was used to calculate heterogeneity as a percentage in the subgroup analysis.

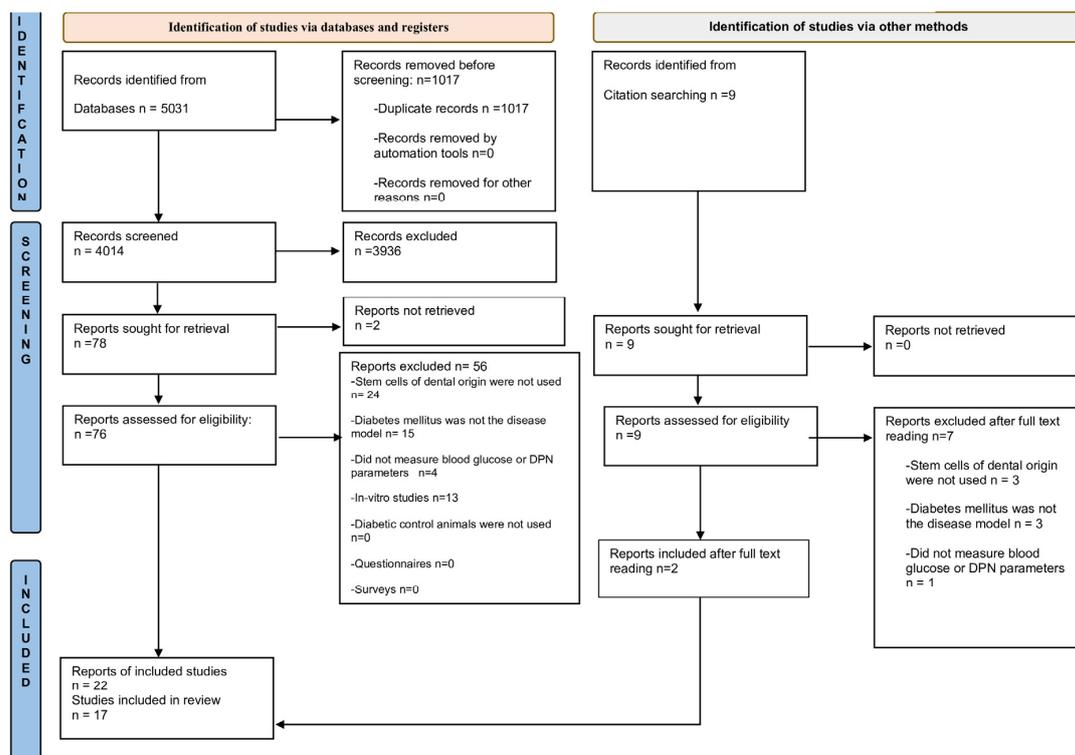
### 2.8.2. Publication Bias

If 10 or more studies provided data in the meta-analysis, the studies were assessed by funnel plot inspection for potential publication bias. Using standard error of the observed outcomes, Egger's regression [44] and Begg and Mazumdar rank correlation [45] tests were performed to provide quantitative assessments of funnel plot asymmetry.

## 3. Results

### 3.1. Study Selection

In total, the database search yielded 5031 records (Figure 1). After removing duplicate records, the abstracts of 4014 articles were screened for potential eligibility, and 76 studies were selected for full text reading. Two articles were retrieved from the reference lists of included studies. In all, 17 animal studies were included in the review.



**Figure 1.** Flow diagram for selection of studies. Flow diagram template from: Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021, 372, n71. doi: 10.1136/bmj.n71. For more information, visit: <http://www.prisma-statement.org/> (accessed on 9 July 2023) [22].

Following a broad search using MeSH terms and free text, as described previously, and when Hooijmans' search filters for animal studies [23] were not used, a single clinical study [6] in which DSCs were used to treat diabetic patients was retrieved. This is included in the review for the purpose of comparative analysis to determine the scope of clinical translation to humans.

### *3.2. Characteristics of Animal Studies*

The descriptive analysis of included animal studies is presented in Table 1. Seven studies were conducted in Japan [13,46–54]; four in China [9,14,55–57]; three in Egypt [58–61]; two in India [17,62]; and one in Brazil [63].

**Table 1.** Descriptive analysis of animal studies.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
<b>SUBSET 1 Stem cells from human exfoliated deciduous teeth (SHED)</b>							
Kanafi et al., 2013 [62]	Male BALB/c mice.  T1DM was induced by intraperitoneal dose of STZ.  SHED from deciduous teeth.  Dental pulp stem cells (DPSCs) from extracted teeth of human adults for in vitro experiments.	6–8 weeks old.	10 weeks after transplantation of islet-like cell clusters (ICCs) from SHED; 2 weeks after graft removal.	Subcutaneous (SC) transplantation of macro capsules.  8 weeks following transplantation, macro capsules were removed to assess effect of removal.  1000 SHED cells packed in each macro capsule.	In vitro: flow cytometry following isolation of DPSCs and SHED from human teeth; adipogenic and osteogenic differentiation, rate of proliferation, insulin release. Immunohistochemistry, reverse transcriptase polymerase chain reaction.  In vivo: blood and urine glucose, body weight (BW) every 48 hours; histopathology of pancreas following sacrifice.	In vitro: Both DPSCs, SHED showed adipogenic and osteogenic differentiation; positive for stromal markers (CD34, CD90 etc.). Proliferative ability greater in SHED than DPSC. Increased expression of insulin on 10th day.  In vivo: No graft rejection in any mouse. Diabetic mice with islet-like cell (ICC) transplantation returned to normoglycemia and normal level of glucose in urine by second week of transplantation, which was maintained for 10 weeks after transplantation and 2 weeks after graft removal.  BW improved. Morphology of islets of Langerhans cells improved. Diabetic mice without SHED transplantation showed hyperglycemia and reduced BW.	In vitro experiment demonstrated that SHED showed greater differentiation and proliferative ability than DPSCs.  In vivo experiment was conducted with ICCs from SHED only.
Izumoto-Akita T et al., 2015 [51]	Male C57BL/6J mice induced T1DM by daily intraperitoneal injection of Streptozotocin (STZ) for 5 days, or a single high dose of STZ.  SHED from 6–12-year-old patients.  Bone marrow mesenchymal stem cells (BMMSCs) from 20–22-year-old patients.	10 weeks old.	3 weeks after treatment ended, 5 weeks from 1st STZ dose.	Intravenous (IV) administration of 1 mL twice daily dose of conditioned medium, i.e., SHED-CM (or 1 mL BMMSC-CM or 24 nmol/kg exendin-4 (Ex-4) -type of incretin that reduces glucose and stimulates insulin production) for 5 days during STZ administration; Thereafter, intraperitoneal administration for 9 days (14 days total) or 3 days following a single high dose of STZ.	In vitro: immunofluorescence studies on the effect of SHED-CM on mouse pancreatic B cells; insulin secretion assay.  In vivo: random plasma glucose at the same time every day for 14 days.  Intraperitoneal glucose tolerance test (IPGTT) on 27th day after 16 h fasting; BW, histopathology of pancreas following sacrifice 3 weeks after treatment.	In vitro: number of necrotic pancreatic cells reduced when treated with SHED-CM; significantly greater effect noted in SHED-CM compared to that of BMMSC-CM and Ex-4.  In vivo: random plasma glucose was reduced on 12th day of treatment; IPGTT showed insulin increased in mice treated with SHED-CM.  No change in BW.  Number of insulin-producing $\beta$ -cells increased with SHED-CM administration.	Despite the high dose of SHED-CM administered, no hematuria or animal deaths caused by administration were reported to occur.  The authors suggested that secreted factors of SHED resulted in fewer complications than transplantation of whole SHED cells and required no immunosuppressive agents.

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
Rao et al., 2019 [55]	<p>Male Goto-Kakizaki (GK) rats as test group; Male Wistar rats as controls.</p> <p>T2DM induced by high fat diet for 2–4 weeks.</p> <p>SHED from 6–8-year-old patients.</p> <p>BMMSCs from 16–20-year-old patients from bone marrow aspirate following 3rd molar extractions.</p> <p>6 rats were transplanted with green fluorescence protein (GFP) GFP-SHED or GFP-BMMSCs for cell tracking in kidneys.</p>	12 weeks old.	<p>For GK rats: 8 weeks after administration of stem cells (i.e., 8–10 weeks from start of experiment); for GFP-SHED and GFP-BMMSC rats: 2, 4, and 8 weeks after administration of SHED or BMMSCs; for Wistar rats: 10 weeks from baseline.</p>	Administered $4 \times 10^6$ cells (SHED or BMMSCs) per animal via tail vein.	<p>In vitro: flow cytometry for surface marker profiles; adipogenic and osteogenic differentiation. Effect of SHED and BMMSCs on epithelial–mesenchymal transition (EMT) caused by advanced glycation end products (AGE).</p> <p>In vivo: BW, fasting, and non-fasting glucose every week; serum triglycerides, IL1, IL10, TNF-<math>\alpha</math>, Hepatocyte growth factor (HGF); renal function tests, renal histological and immunohistochemistry following sacrifice.</p>	<p>In vitro: SHED and BMMSCs showed fibroblast-like morphology. Both showed adipogenic and osteogenic differentiation.</p> <p>In vivo: fasting glucose decreased with SHED and BMMSCs; non-fasting reduced at 2 weeks with BMMSCs, and at 2,3, and 7 weeks in SHED group.</p> <p>Serum triglycerides, urinary albumin, and kidney to body weight ratio remained stable with SHED and BMMSCs as compared to diabetic group with no SC treatment.</p> <p>IL1, TNF-<math>\alpha</math> reduced in both the SHED and BMMSC group; IL10, and HGF increased in both groups compared to the no treatment group; improved renal morphology such as reduced glomerulosclerosis and tubular dilatation seen in treatment with both types of SCs.</p>	<p>Results suggest that both BMMSCs and SHED are effective in treating diabetic nephropathy, although SHED appeared to have more sustained long-lasting effects than BMMSCs.</p> <p>Authors attributed the improvement in renal morphology and local inflammation to the local engraftment (homing) of SHED in kidneys.</p> <p>Study used GK rats, a lean Type II DM model characterized by glucose intolerance.</p>

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
<p>↳ Rao et al., 2019 [56]</p>	<p>Male GK rats as test group, 8 Male Wistar rats as controls.</p> <p>T2DM induced by high-fat diet.</p> <p>SHED from 6–8-year-old patients. Study does not mention if teeth were carious/infected and extracted.</p> <p>Human BMMSCs from commercially available source.</p>	12 weeks old.	<p>8 weeks after SC administration.</p> <p>12 weeks from start of experiment.</p>	Administered $4 \times 10^6$ cells (SHED/BMMSCs) per animal via tail vein.	<p>In vitro: adipogenic and osteogenic differentiation.</p> <p>In vivo: BW, fasting, and non-fasting blood glucose, insulin release test (IRT), homeostatic model assessment for insulin resistance (HOMA-IR).</p> <p>Pancreatic histology and immunohistochemistry.</p> <p>Liver histology and presence of SCs in liver.</p> <p>Quantitative real time (RT-PCR) and Western blotting of liver.</p>	<p>SHED differentiated into adipogenic and osteogenic cells.</p> <p>BW increased during and after treatment but was less than that in normal rats.</p> <p>FBG and non-FBG were less in treatment groups than in PBS group but higher than in normal (non-diabetic) group. No difference b/w SHED and BMMSC groups. HOMA-IR increased in SHED and BMMSC groups.</p> <p>Morphology of pancreatic islet cells improved with SHED as well as BMMSC treatment as compared to vehicle group.</p> <p>Glycogen storage improved in liver with stem cell treatment, whereas in vehicle group, glycogen reduced as compared to normal hepatic cell morphology.</p> <p>PBS group had lower pancreatic beta function values than normal group, which improved in SC groups. SHED and human BMMSCs found in liver in SC groups.</p> <p>RT-PCR showed that T2DM-induced increase in enzymes was reversed with SC administration.</p>	<p>Homing of SCs were found in liver but not in pancreas, suggesting that IV form of administration and impaired liver may cause migration of SCs to distant organs other than the pancreas.</p> <p>Authors suggest that DSCs may cause improvement in parameters by improving B-cell function.</p>
<p>Xie et al., 2019 [14]</p>	<p>CK male rats as test group, male Wistar rats as controls.</p> <p>T2DM induced by high-fat diet.</p> <p>Retained deciduous teeth extracted from 6–10 year old patients.</p>	10 weeks old.	12 weeks after administration of SHED, about 20 weeks from start of experiment.	<p><math>1 \times 10^7</math> SHED transplanted into caudal vein by IV infusion, and repeated once after 2 weeks.</p>	<p>In vitro: flow cytometry for surface markers; differentiation into multiple cell lineage.</p> <p>In vivo: mechanical hyperalgesia by calculating Paw Withdrawal Mechanical Threshold (PWMT) using Dixon's up and down method and Von Frey hairs (VFH) on hind paw; capillary-muscle ratio in soleus muscle, intra-epidermal fiber density (IENFD) in foot pads. Protein expression in skeletal muscle after sacrifice.</p>	<p>In vitro: lack of CD45 surface marker (characteristic of hematopoietic cells), differentiated into osteoblasts, chondroblasts, and odontoblasts.</p> <p>In vivo: SHED found around skeletal muscle bundles. PWMT values increased after 6 weeks in SHED group and were sustained for 2 weeks thereafter; IENFD and morphology of sciatic nerve fibers improved; increase in skeletal muscle capillary density with SHED treatment compared to saline administration group.</p>	<p>Source of stem cells were extracted deciduous teeth.</p> <p>Authors attributed amelioration of diabetic neuropathy to IV infusion for successful homing in skeletal muscles.</p>

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
Miyura-Yura et al., 2020 [50]	Male C57BL/6 Mice.  Induced T1DM by 150 mg/kg intraperitoneal injection of STZ.  SHED from 6–12-year-old patients.	5 weeks old for induced DM study.  Dorsal root ganglions from healthy 4–6-week-old mice.	4 weeks after SHED-CM treatment, 16 weeks after induction of DM.	100 µL SHED-CM administered into unilateral soleus muscle 12 weeks after DM induction, twice a week for 4 weeks.	In vitro: neurite outgrowth in dorsal root ganglion of mice; SHED effect on cell viability of human umbilical vein endothelial cells (HUVECs from cell bank).  In vivo: BW, blood glucose; thermal plantar test, motor and sensory nerve conduction velocity (MNCV, SNCV), intra-epidermal nerve fiber ratio (IENFD), blood flow sciatic nerve (SNBF), capillary-muscle fiber ratio.	Dorsal root ganglion neurites were longer with SHED in vitro.  SHED-CM did not affect glucose levels or body weight of diabetic mice. Von Frey tests showed that thermal sensitivity did not reduce with SHED-CM. SNCV was ameliorated, but MNCV, IENFD did not improve with SHED-CM.  SHED-CM increased capillary-muscle density ratio and improved blood flow in treated side as compared to untreated side of diabetic mice.	Study suggests soluble factors from SHED caused neurite outgrowths, increased number of capillaries, and blood flow in skeletal muscle, which improved neural function.  Treatment with SHED started after 12 weeks of DM with advanced stages of neuropathy, resulting in no improvement in thermal sensitivity, MNCV and IENFD, indicating that early intervention might be more effective.  Study suggests that stem cells may not be as effective in animals of a different species.
Xu et al., 2020 [57]	Male Sprague Dawley (SD) rats.  T2DM was induced in rats which were fed with high-fat diet for 8 weeks, and then administered with a single intraperitoneal dose of STZ.  Rats were divided into five groups: stem cell therapy only (SC); stem cells and hyperbaric oxygen (SC+ HBO); hyperbaric oxygen only (HBO); diabetic control group (DM); and non-diabetic normal rats (NC).	Age of rats at start of experiment was unclear.	6 weeks after treatment with either SHED or hyperbaric oxygen (HBO) or combined SHED and HBO treatment.	Rats administered 0.5 mL SHED through caudal veins in first and third week after induction of T2DM. Fourth group consisted of normal rats on a normal diet. Rats in normal control and HBO groups were transfused with equal volumes of sodium chloride.  HBO treatment with pure oxygen administered for 1 hour daily for 28 days to rats in HBO or combined SC and HBO groups.	BW, blood glucose serum insulin, HOMA-IR, serum lipid panel.  Insulin and glucagon in pancreatic islets following treatment.  Inflammation; apoptosis in pancreatic cells.  Mental state of rats.	Frequency of urination reduced, BW increased in SC+HBO and SC groups.  Blood glucose reduced and serum insulin increased in SC+HBO and SC groups.  Serum LDL, serum TNF-alpha reduced in these groups as compared to DM control group.  Inflammation and apoptosis was reduced in pancreatic cells in SC+HBO and SC groups.  Mental state of rats was better in SC+HBO and SC groups than in DM groups.	Study showed that SHED caused a decrease in serum lipids, and the change was demonstrated to be earlier in combination with HBO.

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
SUBSET 2 DPSCs derived from humans (hDPSCs)							
Datta et al., 2017 [17]	Male Wistar rats induced with DM by single intraperitoneal injection with 55 mg/kg STZ.  hDPSCs from healthy third molars from 18–40-year-old patients.	16 weeks old.	4 weeks and 8 weeks after hDPSC treatment; 10 weeks and 14 weeks after STZ administration.	6 weeks after DM induction, $1 \times 10^6$ hDPSCs administered IV via lateral tail vein or IM through soleus muscle once or with a second dose 4 weeks after the first dose.	In vitro: adipogenic and osteogenic differentiation.  In vivo: blood glucose, BW, thermal hyperalgesia by tail immersion test; grip strength; sciatic nerve conduction; immunohistochemistry following sacrifice; plasma proteins.	Positive for stromal cell markers; adipogenic and osteogenic differentiation.  In vivo: decrease in BW in diabetic rats, BW increased with hDPSC treatment, but decreased after 6 weeks of IV treatment; blood glucose levels were reduced to normal with IM and IV administrations of hDPSCs.  Tail flick test showed thermal sensitivity was reduced with treatment across groups treated with hDPSCs. Grip strength improved more in IV than IM groups, although with IM repeat doses, the grip strength increased more than with a single dose at 8 weeks.	DPSCs caused a reduction of IL-6, TNF- $\alpha$ and increased TGF- $\beta$ . Repeat IM doses of hDPSCs caused an increase in arachidonic acid.  The study suggests that repeat doses of IM transplantation are a more effective long-term option to treat DPN, which may be attributed to the presence of SCs within the soleus muscle.
Al-Serwy et al., 2021 [58]	Male Sprague Dawley (SD) rats.  T1DM induced by STZ by intraperitoneal route.  hDPSCs from adult human impacted third molars.	6–8 weeks old.	28 days after hDPSC transplantation.	Group 1: normal control rats.  Group 2: untreated diabetes induced rats.  Group 3: diabetic rats treated with $1 \times 10^6$ DPSCs by IV administration.	Fasting blood glucose, glucose tolerance test, parotid gland weight and histology, salivary flow rate, caspase-3.	Blood glucose reduced with DPSC transplantation.  Parotid gland weight and salivary flow improved with DPSCs.  Caspase-3 reduced and VEGF upregulated with DPSCs.	Presence of transplanted DPSCs in STZ-injured parotid gland demonstrates the migratory ability of stem cells towards injured tissue.

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
El-Kersh et al., 2020 [59]	Male SD rats.  Healthy impacted third molars from adult patients.  T1DM induced by intraperitoneal injection of STZ.	6 weeks old.	4 weeks after transplantation; 5 weeks after DM induction.	Rats were divided into four groups: non diabetic rats, diabetic rats that were given buffer; DM rats that were treated with $1 \times 10^6$ DPSCs by IV; and DM rats that were treated with $1 \times 10^6$ DPSCs via intrapaneacric administration.  For IV group, DPSCs administered into tail vein; for intrapaneacric group DPSC suspension administered into pancreas.	Insulin and C-peptide assay, blood glucose.  Pancreatic immuno- histochemical and histological analyses.	Blood glucose levels in IV group reduced by 7th day following transplantation; in intrapancreatic group, the blood glucose levels were reduced by 14th day after DPSC treatment.  Glucose homeostasis was maintained as established by glucose tolerance tests after 4 weeks of DPSC treatment in both IV and intrapaneacric groups.  Insulin and C-peptide levels were higher in the DPSC treated groups than in the non-treated group.  Pancreatic islet morphology and angiogenesis improved in both intrapaneacric and IV groups.	The study demonstrated that pancreatic function was re- established and maintained for 4 weeks following DPSC treatment.  The study showed that intrapancreatic and IV administrations have comparable therapeutic results in an experimental T1DM model.
Hata et al., 2021 [48]	BALB/cA cl-nu/nu nude male mice.  hDPSCs from impacted third molars from human adults aged 13–23 years.  T1DM induced by single dose of intraperitoneal injection of STZ. Mice that were not treated with STZ were normal controls.	6 weeks old.	16 weeks following transplantation of hDPSCs; 24 weeks after induction of DM.	Intramuscular (IM) transplantation of hDPSCs in saline injected in 10 separate sites in the hind- limb on one side. Saline was injected into the opposite hind limb on the control side.	Neurite outgrowth of mouse dorsal root ganglion (DRG) in vitro.  Blood glucose; body weight (BW).  4 and 16 weeks after transplantation, SNCV, sciatic blood flow, current perception threshold (CPT).  Location of transplanted hDPSCs in gastrocnemius muscle.	BW was reduced in DM mice than in normal control mice, blood glucose was significantly increased in DM mice than in normal control mice.  hDPSCs promoted DRG neurite outgrowth in vitro.  MNCV and SNCV and sciatic blood flow reduced in DM mice in saline injected sides; however, they significantly improved in hDPSC sides at 4 weeks, and were maintained upto 16 weeks post- transplantation.  CPT significantly improved in hDPSC injected side at 4 weeks up to 16 weeks post-transplantation.  hDPSCs were found around muscle bundles of gastrocnemius muscle in hDPSC side and not in saline injected sides 16 weeks post- transplantation.	Longer duration effects of DSCs on DPN were highlighted in this study.

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
<sup>L</sup> Hata et al., 2020 [49]	Male nude mice (BALB/cA]cl-nu/nu).  hDPSCs from impacted third molars extracted from humans 13–23 years of age.  DM induced by intraperitoneal injection of STZ. Mice that did not receive STZ were normal controls.	6 weeks old.	4 weeks following hDPSC trans plantation, 12 weeks after induction of DM.	8 weeks after STZ administration, hDPSCs in saline injected in 10 separate sites in unilateral right hind limb skeletal muscle of all mice.  Saline was injected into the left hind limb muscle on the opposite side as controls.	Body weight (BW), blood glucose.  MNCV, SNCV, SNBF, CPT  Characterization of transplanted hDPSCs.  Capillary-muscle density ratio in gastrocnemius muscle.  Effect of human VEGF and NGF antibodies on hDPSC treatment.	CPT increased in saline injected sides and improved in hDPSC injected sides of mice.  All diabetic mice showed reduced BW and increased blood glucose at the end of experiment.  MNCV, SNCV, and SNBF reduced in saline -injected sides of DM mice compared to normal mice, but were significantly improved in hDPSC-treated sides of diabetic mice.  hDPSCs were found localized around muscles 4 weeks after transplantation, whereas the saline injected sides did not show any hDPSCs.  Human VEGF, NGF expressed in hDPSC sides only in normal and diabetic mice. VEGF and NGF antibodies suppressed hDPSC effects in MNCV and SNCV in hDPSC transplanted sides of mice.  Capillary-muscle ratio reduced in DM mice in vehicle side, but improved significantly in hDPSC side of diabetic mice.	Suppressing effect of human VEGF and NGF antibodies on hDPSCs in DM mice shows that angiogenic and neurotrophic factors are important for hDPSC treatment.  Potential cross effects of human VEGF and NGF antibodies on mouse VEGF and NGF may be present.

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
Ahmed et al., 2021 [60]	Adult male Wistar rats.  Insulin producing cells (IPCs) from hDPSCs derived from human adult teeth.  T1DM induced by single dose of SC injection of STZ. Normal controls were not treated with STZ.	Age of rats unclear.	28 days following IPC transplantation.	Group 1: 10 rats—normal controls.  Group 2: 10 rats—untreated diabetic rats.  Group 3: 10 rats—diabetic rats treated with IPCs from DPSCs generated in the presence of cerium nanoparticles by IV administration (tail vein).  Group 4: 10 rats—diabetic rats treated with IPCs from DPSCs generated in the presence of yttrium nanoparticles by IV.	Blood glucose, serum insulin (INS), hepatic hexokinase, glucose 6 phosphate dehydrogenase (G6PD), location of labeled IPCs in body following transplantation.	Blood glucose reduced and INS increased following IPC administration in both treated diabetic groups compared to diabetic controls.  Hepatic hexokinase and G6PD, which were reduced in induced diabetes, increased in Group 3 compared to untreated diabetic rats. Group 3 and 4 showed increase in G6PD in diabetic rats; however, Group 3 generated greater G6PD activity than Group 4. Transplanted IPCs were located in the pancreas and improved pancreatic morphology.	Conditioned IPCs underwent hypoxia prior to transplantation.
Inada et al., 2022 [54]	40 Male nude F344-NJCl-rnu/rnu rats.  T1DM induced by STZ via intraperitoneal route.  hDPSCs from adult teeth.  Human BMSCs (hBMSCs) obtained from SC bank.  Tacrolimus was injected in diabetic group rats, along with transplants.  Insulin implants were placed in diabetic rats at 12 weeks of age and removed at 16 weeks of age.	9 weeks old.	4 weeks after hDPSC transplantation and at 18 weeks of age.	Group 1: normal control rats.  Group 2: diabetic control rats.  Group 3: rats transplanted with hBMSCs.  Group 4: rats transplanted with 2D hBMSCs.  Group 5: 3D hBMSCs transplanted rats.  Group 6: hDPSC transplanted rats.  Group 7: 2D hDPSC transplanted rats.  Group 8: 3D hDPSC transplanted rats.  Transplants of IPCs from SCs were placed in capsules of the left kidney of each animal; the cells were $5 \times 10^6$ per animal.	Non-fasting blood glucose, glucose tolerance test.  Water consumption.  Immunohistochemistry of kidneys.  Serum human and rat insulin, serum urea, and creatinine.	After insulin implant was removed, water consumption increased in all except the 3D hDPSC group. All groups except for the 3D hDPSC group showed an increase in blood glucose after insulin implants were removed.  Human as well as rat insulin increased in the 3D-hDPSC group.	After measuring serum urea and creatinine levels, it was found that there were no differences between diabetic rats and normal controls. The authors concluded that there were no effects of stem cells on the kidneys.  Since rat as well as human insulin increased in the 3D hDPSC group, it was determined that transplanted IPCs performed an endocrine function and also aided in regeneration of host islet cells.

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
<b>SUBSET 3 human gingival stem cells (hGMSCs)</b>							
Zhang et al., 2017 [9]	<p>Wild type C57BL/6-foxgfb male mice.</p> <p>Induced with T1DM by multiple doses of STZ 40 mg/kg via intraperitoneal route for 5 days.</p> <p>Healthy gingival tissue from third molar extraction sites of Asian male or female patients aged 20–30 yrs. old.</p>	6–8 weeks old.	10 days and 30 days after STZ administration.	<p><math>1 \times 10^6</math> GMSCs (test group) or dermal fibroblasts (group 2) administered via intraperitoneal route at 0, 7, 14, 21, and 28 days after STZ administration.</p>	<p>In vitro: suppressive assay; murine CD4+T cell differentiation.</p> <p>In vivo: non-fasting blood glucose twice a week for 30 days following STZ administration.</p> <p>Histology and Immunohistochemistry of pancreas following sacrifice.</p>	<p>GMSCs have similar morphology to fibroblasts. CD4+T cell differentiation was reduced; GMSCs expressed CD39 and CD73 molecules.</p> <p>DM was delayed with GMSC administration; blood glucose was reduced more than with fibroblasts; DM was not prevented completely with GMSCs.</p> <p>More islet cells stained positive for insulin in the GMSC group, and insulinitis was reduced significantly more than with fibroblasts. IL-17 and interferon-<math>\gamma</math> reduced after GMSC administration.</p>	<p>The study suggests the immunomodulatory mechanism of GMSCs is through CD39 or CD73 signals.</p> <p>T1DM was suppressed due to IL17 inhibition by GMSCs.</p> <p>GMSCs were found homed in pancreas and pancreatic lymph nodes, possibly attributed to intraperitoneal route.</p>
<b>SUBSET 4 Stem cells derived from human PDL (hPDLSCs)</b>							
Aly et al., 2022 [61]	<p>Adult male Sprague Dawley (SD) rats.</p> <p>IPCs from PDLSCs from healthy impacted human third molars and from human DPSCs from third molars (aged 16–24 years).</p> <p>Differentiation into IPCs was induced by Laminin 411.</p> <p>T1DM induced with single SC dose of STZ.</p>	Age of rats unclear.	28 days after transplantation.	<p>Group 1: normal controls.</p> <p>Group 2: untreated diabetic controls.</p> <p>Group 3: diabetic rats treated with <math>5 \times 10^6</math> IPCs from human DPSCs by IV route.</p> <p>Group 4: diabetic rats treated with <math>5 \times 10^6</math> IPCs from human PDLSCs by IV route.</p>	<p>Blood glucose, serum insulin (INS), C-Peptide (CP)</p>	<p>Blood glucose reduced with IPC transplantation from both human DPSCs and PDLSCs.</p> <p>INS and CP increased with IPCs from both sources.</p>	<p>The study demonstrates the efficacy of IPCs from PDLSCs, as well as DPSCs in regulating glucose and insulin in diabetic rats.</p>

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
<b>SUBSET 5 DPSCs derived from teeth of rats (rDPSCs)</b>							
Hata et al., 2015 [46]	Male SD rats induced with T1DM by a single dose of 60 mg/kg STZ intraperitoneal injection.  DPSCs from incisors of 6-week-old male SD rats or green fluorescent protein (GFP) SD rats.	6 weeks old.	4 weeks after transplantation of rDPSCs; 12 weeks from time of DM induction.	1 × 10 <sup>6</sup> cells per limb (either freshly isolated or partly frozen for 6 months) of DPSCs injected into the unilateral skeletal muscle of hind limb while 1 mL saline administered similarly in the other hind limb, 8 weeks following induction of T1DM.	In vitro: assessment of cell surface markers CD34, CD49d, and CD45. Differentiation of fresh and frozen DPSCs.  In vivo: 4 weeks after DPSC administration, MNCV, SNCV, SNBF, IENFD, capillary-muscle fiber ratio, and location of DPSCs from GFP rats.	DPSCs were spindle shaped and expressed CD29 and CD90. Both fresh and cryopreserved DPSCs showed adipogenic and osteogenic differentiation. DPSCs expressed VEGF and bFGF.  MNCV, SNCV, SNBF and IENFD increased in fresh as well as in cryopreserved DPSC injected hind limbs; vascular endothelial cell - muscle ratio increased with DPSCs; DPSC engrafted around skeletal muscle and did not differentiate into adipocytes or osteoblasts.	Transplanted DPSCs differentiated into PECAM-1 positive vascular endothelial cells, as indicated by GFP stained cells.  The study showed that the proliferative ability of frozen DPSCs was similar to that of freshly isolated DPSCs.
↳ Omi et al., 2016 [47]	Male SD rats induced with T1DM by intraperitoneal injection of 60 mg/kg STZ.  DPSCs from mandibular incisors of 6-week-old normal male SD rats or GFP transgenic SD rats.	6 weeks old.	4 weeks after transplantation of DPSCs; 12 weeks from induction of DM.	8 weeks following induction of DM, administration into unilateral hind limb with 1 × 10 <sup>6</sup> DPSCs and 1 mL saline in opposite hind limb.	In vitro: morphology and characterization of DPSCs; adipogenic and osteogenic differentiation.  In vivo: MNCV, SNCV, SNBF; histology following sacrifice; no monocytes or macrophages in sciatic nerves; IL10, VEGF, bFGF.	In vitro: DPSCs expressed angiogenic factors such as VEGF, bFGF, NGE.  In vivo: BW, blood glucose was not affected by DPSC treatment. Monocytes/macrophage numbers were reduced; delayed MNCV, SNCV, and SNBF was improved with DPSCs in treated hind limb; TNF-α was reduced and IL10 increased in sciatic nerve in treated side.	The study suggests that DPN could be related to inflammatory processes, as reduction of macrophages and pro inflammatory factors TNF-α, and an increase in IL-10 occurred with concomitant amelioration of DPN following DPSC administration. The study showed that angiogenic factors may also play a role in the treatment of DPN.
Omi et al., 2017 [13]	Male SD rats induced with T1DM by intraperitoneal injection of STZ 60 mg/kg.  DPSCs harvested from incisors of 6-week-old SD rats.  Dorsal root ganglions (DRG) from 8-week-old mice.	6 weeks old.	4 weeks after DPSC administration; 52 weeks after induction of DM.	48 weeks following DM induction, 1 × 10 <sup>6</sup> DPSCs administered into unilateral hind limb; saline was injected on opposite hind limb as the control.	In vitro: differentiation potential, neurite outgrowth of DRG, Schwann cell viability.  In vivo: MNCV, SNCV, SNBF, current perception threshold (CPT); capillary density, nerve fiber density following sacrifice.	DPSCs differentiated into adipocytes and osteocytes and chondrocytes, promoted neurite outgrowth of DRG Schwann cell viability and myelin growth.  MNCV, SNCV, SNBF, IENFD, and CPT improved in DPSC treated limb, DPSCs caused increase in the expression of NGE, bFGF; capillary density and myelin thickness of sural nerve was increased in treated side.	The experiment was conducted in rats which were induced with long-term DM (48 weeks).  The study suggests that DPN was reduced due to the effects of DPSCs on myelin thickness of nerves.

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
Makino et al., 2019 [52]	Male SD rats induced with T1DM by intra- peritoneal injection of STZ 60 mg/kg.  Conditioned medium of DPSCs (DPSC-CM) derived from incisors of 6-week -old SD rats after sacrifice.	6 weeks old.	4 weeks after DPSC administration; 12 weeks after DM induction.	8 weeks after DM induction by STZ, $1 \times 10^6$ DPSC-CM administered in unilateral hind limb; 1.0 mL/rat saline injected in opposite hind limb.	In vitro: cell proliferation assay with human umbilical vein endothelial cells (HUVEC); differentiation potential.  In vivo: Body weight (BW), blood glucose, MNCV, SNCV, SNBE, IENFD, and immune- histochemistry following sacrifice.	Increased proliferation of HUVECs.  No changes in BW and blood glucose. MNCV, SNCV, SNBE, nerve density increased on DPSC-CM injected hind limb; number of macrophages reduced in sciatic nerve of DPSC-CM treated limb; capillary density in skeletal muscle increased with DPSC-CM, but was unaffected in sciatic nerves in hind limb.	DPSC-CM contains VEGF; hence, its use may be contra-indicated in patients with risk of diabetic retinopathy.
<sup>†</sup> Kanada et al., 2020 [53]	Male SD rats.  DPSCs harvested from incisors of 6-week-old male green fluorescent protein GFP transgenic SD rats.  Intraperitoneal injection of STZ used to induce DM.	6 weeks old.	4 weeks after DPSC administration; 12 weeks after DM induction.	DM induced rats were either injected with saline, DPSCs or secretory factors of DPSCs (DPSC-SF) in the skeletal muscles of unilateral hind limb.  Rats that were not induced with DM were included in the normal control group.	BW, blood glucose.  SNCV, MNCV SNBE, intraepidermal nerve fiber density (IENFD).  Capillary-muscle ratio in skeletal hind limbs.  Characterization of secretory factors of DPSCs used in the study.	DM-induced rats showed lower BW and higher blood glucose levels than non-DM rats. Neither DPSCs nor DPSC-SF showed significant improvement in the BW or blood glucose values in DM rats.  DPSCs and DPSC-SF improved SNCV, MNCV, sciatic nerve blood flow and IENFD values in the hind limbs that were treated as compared to saline injected DM rats.  Muscle volume and capillary-muscle ratio improved with DPSC and DPSC-SF administration.	No significant difference between the effects of DPSCs and DPSC-SF administration.  The study design demonstrated that SC administration results were limited to at or near the site of administration.  VEGF, NGE, and IL-1 $\beta$ were identified as some of the secretory factors of DPSCs.

Table 1. Cont.

Study	Animal Used in Study/ Method of Disease Induction/Source of Stem Cells	Age of Animal at Start of Experiment	Time of Sacrifice	Route/Mode of Stem Cell Administration	Parameters Assessed	Results	Additional Comments
SUBSET 6 DPSCs derived from teeth of mice (mDPSCs)							
Guimarães et al., 2013 [63]	Female C57BL/6 mice induced T1DM by 3 daily intraperitoneal injections of 80mg/kg STZ.  DPSCs from mandibular incisors of male enhanced GFP C57BL/6 mice.	8 weeks old.	30 days and 90 days after first STZ dose.	10 days after first STZ induction of DM, administration of $1 \times 10^6$ DPSCs in each mouse via orbital plexus injection.	BW, weekly blood glucose, proteinuria, glycosuria, urea, histopathological assessment of pancreas and kidneys following sacrifice 30 days after DM induction; tail flick test up to 90 days after 1st STZ dose.	At 21 days after DPSC treatment, blood glucose levels were reduced in diabetic mice; BW was normalized.  Increase in insulin producing pancreatic cells was seen with DPSC treatment. Engraftment of stem cells in the pancreas was observed. Reduced glucose, protein and increased urea levels were found in the urine. Morphologic changes in the kidneys, which were found in non-treatment diabetic mice, such as the loss of the epithelial brush border, were not seen in DPSC-treated mice, and less deposition of glycogen in the tubules was noted in DPSC-treated mice.  Mice developed nociceptive values comparable to those of non-diabetic mice 3 days after DPSC treatment, a result that was maintained throughout the study.	The study showed that DPSCs improved kidney function.  C57BL/6 mice are known to be relatively resistant to nephropathy.  Blood glucose levels increased gradually, in spite of DPSC treatment, suggesting that repeat doses of DPSCs may be required. Donor mice stem cells secreting insulin were found engrafted in the pancreas.

<sup>L</sup> Multiple reports of the same study reporting different outcomes are presented together.

### 3.2.1. Animal Model Characteristics

Streptozotocin (STZ) was used to induce T1DM in 14 studies [9,13,17,46,48,50–52,54,58,60–63]; T2DM was induced using a high-fat diet in 2 studies [14,55], while in 1 study, T2DM was induced in rats which were given a high-fat diet and then administered with STZ [57]. Sprague Dawley (SD) rats were used in six studies [13,46,52,57,58,61]; Goto-Kakizaki (GK) rats were used in two [14,55]; Wistar rats in two [17,60]; Fischer 344 rats (F344-N)cl-rnu/rnu in one study [54]; four studies used C57BL/6 mice [9,50,51,63]; and two used Bagg Albino (BALB/c) mice [48,62]. In 1 study, female mice were used [63], whereas 16 studies used male rats or mice. Animals were sacrificed at a timepoint ranging from 20 days to 16 weeks after the transplantation of DSCs, and 4 to 52 weeks from the induction of diabetes.

### 3.2.2. Dental Stem Cell Selection in Animal Studies

SHED were used in 6 studies [14,50,51,55,57,62], and human DPSCs (hDPSCs) were used in five [17,48,54,58,60]. In one study, MSCs from human gingiva (hGMSCs) were used [9], whereas in another, insulin-producing cells (IPCs) derived from human periodontal ligament stem cells (hPDLSCs) and hDPSCs were used [61]. Rat derived DPSCs (rDPSCs) were used in three studies [13,46,52]; one study used DPSCs derived from the incisors of mice (mDPSCs) [63]. Rodent derived DPSCs were from the same species as the animals used to study DSC effects, although autotransplantation was not performed in any study. Whole SCs were used in 14 studies [9,13,14,17,46,48,54,55,57,58,60–63], and 3 used serum-free conditioned medium containing secretory factors of SHED (SHED-CM) [50,51] or DPSCs (DPSC-CM) [52].

Intravenous (IV) route was used to administer DSCs in seven studies [14,55,57,58,60,61,63], and intramuscular (IM) route was used in five [13,46,48,50,52]. In one study, IPCs obtained from SHED were transplanted subcutaneously [62], while in another [54], IPCs were placed under renal capsules of diabetic animals. Intraperitoneal route was used in one study [9], and in another, IV route was followed by use of intraperitoneal route [51]. In two studies, IV route was compared with other methods of DSC administration such as IM [17] and intrapancreatic routes [59]. One study reported the use of tacrolimus as an immunosuppressant [54]. There were no incidents of graft rejection reported in any study.

### 3.3. Effect of DSCs in Animals

Following the induction of diabetes, blood glucose increased in T1DM and T2DM animals. A decline in blood [9,17,51,54–63] and urinary glucose [62,63] occurred after DSC administration. Serum insulin and C-peptide levels improved [59,61]. Insulin resistance decreased in one T2DM study [57], whereas in another, it increased [56]. Body weight, which had markedly reduced with STZ administration, increased following DSC administration [17,57,62,63].

DPN parameters such as SNCV, MNCV, and sciatic nerve blood flow (SNBF) improved after DSC administration [13,46,48,52]. Intra-epidermal nerve fiber density (IENFD) increased [13,14,46,52], and current perception threshold (CPT) values improved [13,48]. Hyperalgesia, measured by paw withdrawal mechanical threshold (PWMT), Von Frey hairs on hind paw test (VFH) [14] and tail flick test [63] reduced with DSC administration. Twelve weeks after T1DM induction, hypoalgesia, observed in small nerve fibers, was ameliorated following hDPSC transplantation [49]. Thus, early onset hyperalgesia and late onset hypoalgesia were reversed [14,49,63]. Thermal sensitivity, evaluated using the tail immersion test, as well as grip strength improved after hDPSCs administration [17]. In one study, rats which had been induced with T1DM 48 weeks prior to rDPSC administration, showed improvement in DPN parameters [13].

A restoration of islet structure was observed [9,57,59,61–63]. Insulinitis in T1DM animals reduced after administration of hGMSCs [9]. DSCs engrafted and transdifferentiated into insulin secreting cells in the pancreas following various routes of administration [9,51,56,63]. Ki67, a biomarker for cell differentiation, revealed an increase in the proliferation of islet cells [57,59]. The ratio of insulin secreting  $\beta$  cells to total pancreatic cell mass increased [9,59].

hDPSC transplantation resulted in the downregulation of caspase-3, a protease which mediates cell apoptosis, and there was an increase in beta cell mass [58].

In T2DM rats, glycogenesis and glycogen storage increased, and glycolysis reduced following SHED administration [56]. T2DM induced renal tubular dilatation and glomerulosclerosis [55], and T1DM-induced loss of the renal tubular epithelial brush border was reversed [63] following IV administration of DSCs. Inada et al. (2022) reported the presence of engrafted IPCs in the kidneys, 4 weeks after transplantation underneath the renal capsule; however, kidney function was not affected [54]. hDPSCs homed into STZ-induced injured parotid gland tissue after administration via the tail vein [58]. Parotid gland weight and salivary flow increased [58].

DPSCs differentiated into vascular endothelial cells [46]. The capillary-muscle ratio improved in muscles in which DSCs had been administered [13,14,46,49,50,52]. Myelin thickness and area increased [13], and the axonal circularity of sural nerves [47] improved. Intriguingly, hGMSCs engrafted mainly in the mesenteric and pancreatic lymph nodes, and to a lesser extent, in the pancreas, 4 weeks following intraperitoneal administration [9].

A reduction in C-reactive protein (CRP), TNF- $\alpha$ , IL1, IL6, IL17, and interferon- $\gamma$  was observed after DSC administration [9,17,47,55,57]. Arachidonic acid [17], transforming growth factor- $\beta$  (TGF- $\beta$ ) [17], and IL10 [47,55] increased. Vascular endothelial growth factor (VEGF) [47,49,58], nerve growth factor (NGF), and basic fibroblast growth factor (bFGF) levels improved [13,49,53].

Compared to IV, repeat IM doses were more effective in improving DPN measures in one study [17]. The effects on blood glucose were similar in intrapancreatic and IV administrations of hDPSCs in another study [59]. Improvements in DPN parameters were comparable with DPSCs and SFs of DPSCs [53]. More effective glycemic control was observed with human DSCs than with human BMMSC treatment [51,54].

### 3.4. Characteristics of the Clinical Study and Effects of DSCs in Humans

The clinical study was a proof of concept study conducted in China. SHED, which had been isolated from exfoliated teeth of donors, were used in T2DM patients [6]. A total of 24 patients, 45–65 years of age, were enrolled [6]. Daily insulin requirements reduced during the 6-week treatment period and the 12 months of follow up. Fasting blood glucose levels were significantly lower than those at baseline during treatment, but not at the end of follow up [6]. Post-prandial serum C-peptide significantly increased after the treatment period compared to baseline, but the increase was not statistically significant at the end of follow up.

### 3.5. Internal Validity of Animal Studies

The SYRACLE tool, by Hooijmans et al. (2014) [24], consists of six categories: selection, performance, detection, attrition, and reporting biases. The sixth category includes other potential sources of bias, such as pooling of drugs and unit of analysis errors. In general, details regarding study design, such as randomization during allocation, were not mentioned or were unclear (Table 2). Further, the health status of the teeth used to harvest DSCs should be reported, as carious pulpal involvement and history of invasive procedures may affect DSC viability [20,64].

**Table 2.** Internal validity in animal studies. The table is based on the SYRACLE (Systematic Review Center for Laboratory Animal Experimentation) tool by Hooijmans et al. (2014) [24], which in turn is based on the Cochrane RoB (Risk of bias) tool [25], to assess the risk of internal bias. The tool includes six main categories of bias including selection, performance, detection, attrition, reporting, and other sources of bias, such as unit of analysis errors and pooling drugs or contamination. Each category consists of components or ‘domains’ (ten in all). Hooijmans et al. formulated a series of questions under each domain that helps reviewers to ascertain the risk of bias in the studies. If a signaling question is answered with a ‘no’, it is indicative of high risk of bias; if it is answered with a ‘yes’, it indicates a low risk of bias, and an answer of ‘unclear’ indicates an unclear risk of bias in that domain. In this table, ‘Low’ means that that all the signaling questions in that domain were answered with a ‘yes’ and hence, the risk in that domain is deemed low by the reviewers. ‘High’ means that at least one signaling question related to the domain was answered with a ‘no’ and is hence deemed to have a high risk of bias in that domain and category. ‘U’ signifies that the risk of bias in that category is unclear, because some or all answers to the signaling questions in that domain are unclear.

Study	Selection Bias—Sequence Generation	Selection Bias—Baseline Characteristics	Selection Bias—Allocation Concealment	Performance Bias—Random Housing	Performance Bias—Blinding	Detection Bias—Random Outcome Assessment	Detection Bias—Blinding	Attrition Bias—Incomplete Outcome Data	Reporting Bias—Selective Outcome Reporting	Other—Other Types of Bias
Guimerães et al., 2013 [63]	U	Low	U	U	U	U	U	U	Low	U
Kanafi et al., 2013 [62]	U	Low	U	U	U	U	U	U	U	U
Hata et al., 2015 [46]	U	U	U	U	U	U	U	U	U	High ***
<sup>‡</sup> Omi et al., 2016 [47]	U	U	U	U	U	U	U	U	U	High ***
Izumoto-Akita et al., 2015 [51]	U	Low	Low	U	High *	U	U	Low	U	U
Omi et al., 2017 [13]	U	Low	U	U	U	U	U	U	U	High ****
Datta et al., 2017 [17]	U	Low	U	U	U	U	U	Low	U	High ^
Zhang et al., 2017 [9]	U	Low	U	U	U	U	U	U	Low	Low
Rao et al., 2019 [55]	U	Low	U	U	High *	U	U	Low	Low	Low
<sup>‡</sup> Rao et al., 2019 [56]	U	Low	U	U	High *	U	U	Low	Low	Low
Makino et al., 2019 [52]	U	Low	U	U	High *	U	U	U	U	U
<sup>‡</sup> Kanada et al., 2020 [53]	U	Low	U	U	High *	U	U	U	U	U
Xie et al., 2019 [14]	Low	Low	U	U	High *	U	U	Low	U	Low
El-Kersh et al., 2020 [59]	U	Low	U	U	High *	U	U	Low	High **	U
<sup>‡</sup> Al-Serwy et al., 2021 [58]	U	Low	U	U	High *	U	U	Low	High **	U
Miyura Yura et al., 2020 [50]	U	U	U	U	U	U	U	U	U	U
Hata et al., 2021 [48]	U	Low	U	U	High *	U	U	U	U	High ***
<sup>‡</sup> Hata et al., 2020 [49]	U	Low	U	U	High *	U	U	U	U	High ***

Table 2. Cont.

Study	Selection Bias— Sequence Generation	Selection Bias— Baseline Characteristics	Selection Bias— Allocation Concealment	Performance Bias— Random Housing	Performance Bias— Blinding	Detection Bias— Random Outcome Assessment	Detection Bias— Blinding	Attrition Bias— Incomplete Outcome Data	Reporting Bias— Selective Outcome Reporting	Other— Other Types of Bias
Xu et al., 2020 [57]	U	Low	U	U	High *	U	U	U	U	U
Ahmed et al., 2021 [60]	U	Low	U	U	U	U	U	Low	High **	U
Aly et al., 2022 [61]	U	Low	U	U	U	U	U	U	High **	U
Inada et al., 2022 [54]	U	U	U	U	U	U	U	U	High **	U

\* The author(s) who designed the experiment also conducted and analyzed the results of the experiment. According to the SYRCL tool, this could potentially lead to inadequate blinding, and hence, these studies were deemed to be at high risk for performance bias. \*\* Study did not report changes in body weight corresponding to changes in blood glucose during the experiment, which reviewers ascertained to be a key outcome measure for a T1DM animal model. \*\*\* Studies which used one hind limb of each diabetic animal to test the intervention and the opposite hind limb of the same animal as a diabetic control were deemed to be at risk for unit of analysis errors. ^ Insulin was subcutaneously administered throughout the course of the experiment to simulate sustained diabetes mellitus in humans. This may have potentially influenced the results of the study (pooling drugs/contamination based on SYRCL). ^\*\*\* Insulin was subcutaneously administered once per month 8 weeks after STZ administration to simulate long-term diabetes and prevent excessive hyperglycemia. Additionally, the study used one hind limb of each animal for the intervention and the contra lateral hind limb of the same animal to administer saline as diabetic control. This may have led to contamination/pooling drug and unit of analysis errors. <sup>l</sup> Multiple reports of a single study are presented together. The analysis was performed for all reports. Assessments of reports which demonstrated greater risk of bias in any category than other report(s) of the same study are presented in the table to represent each study. If all reports of the same study demonstrated similar risk of bias, the most recent report was used to represent the study.

In some studies [14,48,51,53,56–58], the author(s) who designed the protocol also conducted the experiments and interpreted the results. According to Hooijmans et al. [24], this may create a risk for performance bias, due to inadequate blinding of investigators from knowing which intervention was provided to each animal. Some studies assessing the effects of DSCs on blood glucose in T1DM as an outcome measure [54,58,60,61] did not report changes in body weight during and after treatment. It is imperative that body weight is measured, particularly in T1DM animal models, to ensure that glucose does not decrease because of toxic effects of the intervention or loss of appetite from stress [12]. In some studies, the hind limb on one side was used for administration of DSCs, and the contralateral hind limb of the same animal was used as a control [13,47,48]. This could have led to unit of analysis errors, as potential systemic effects were not considered, and it was uncertain whether or not DSCs affected the side of the animal in which their effects were not intended.

Insulin was administered through the course of the experiments in two studies to prevent excessive hyperglycemia and simulate long-term diabetes mellitus occurring in humans [13,17]. This could have confounded the true effects of DSCs on blood glucose. For instance, in another study [54], insulin implants were placed in animals and were removed 2 weeks before the end of the experiment. While on extrinsic insulin, blood glucose levels decreased in all SC treated diabetic groups and were comparable to those in the normal control group [54]. However, after insulin implant removal, blood glucose increased in all SC treated diabetic groups, except in the hDPSC group which had undergone a 3D differentiation protocol prior to administration [54].

### 3.6. External Validity of Animal Studies

The extent to which data from animal studies can be reliably applied in humans was analyzed (available as Supplementary Table S4).

It is important that studies represent heterogeneity in human populations [65,66]. Rodents of identical strain, species, age, and gender were used in the animal studies. In the clinical study [6], the exclusion criteria precluded the inclusion of patients that diabetes often manifests in, such as pregnant women and patients with co-morbidities [11,12].

The use of an appropriate study population better reflects the disease in humans [65,66]. The age of animals used in T1DM studies ranged from 5 to 16 weeks at the time of disease induction; the age of animals used in T2DM studies ranged from 10–12 weeks. In addition, only one animal study [63] used female rodents. On the other hand, it was unclear how many females and males were enrolled in the clinical study [6].

Omi et al. (2017) established a diabetic rodent model 48 weeks prior to rDPSC administration [13]. Since the lifespan of SD rats ranges between 2.5 and 3.5 years [67,68], this study can be considered to be a long-term diabetic rodent model. The clinical study [6] population was an appropriate representation of a chronic form of diabetes, as the patients had been diagnosed with T2DM for more than 5 years and were using insulin for not less than one year.

It is imperative that DSC therapy is not toxic and is, at the same time, effective for glycemic control and DPN. The definitions for diabetes are different for mice, since they tend to have higher blood glucose concentrations than humans [69]. Furthermore, organs such as the pancreas, liver, kidneys, brain and muscles were removed for histological study after sacrifice in the animal studies. In contrast, postmortem tests are not routinely performed in humans to verify the results of treatment [24].

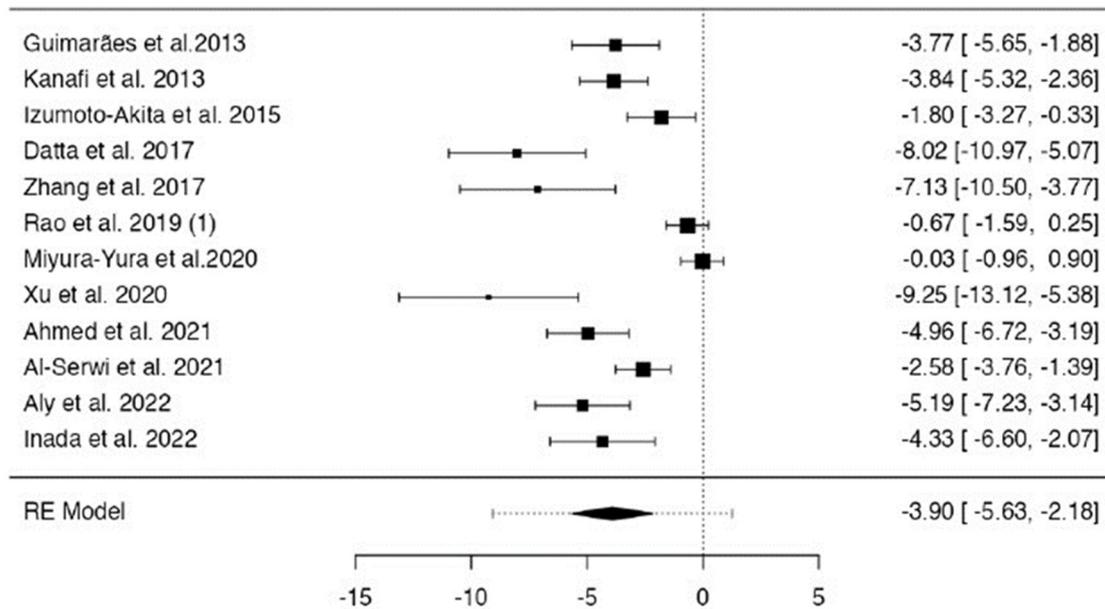
### 3.7. Meta-Analysis

#### 3.7.1. Forest Plot Analysis

##### Effect of DSCs on Blood Glucose

The SMDs of individual studies were within the range of -9.2479 to -0.0270 (Figure 2). The average SMD of the analysis was observed to be -3.905 (95% CI: -5.6330 to -2.177). The average outcome differed significantly from zero ( $Z = -4.9737, p = 0.0004$ ). Hence, the

analysis indicated that there was a significant reduction in blood glucose in favor of DSC administration compared to saline/vehicle.



Random-Effects Model (k = 12)

	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	-3.90	0.785	-4.97	<.001	-5.633	-2.177

Heterogeneity Statistics

Tau	Tau <sup>2</sup>	I <sup>2</sup>	df	Q	p
2.216	4.9123 (SE= 2.9104)	88.59%	11.000	96.419	<.001

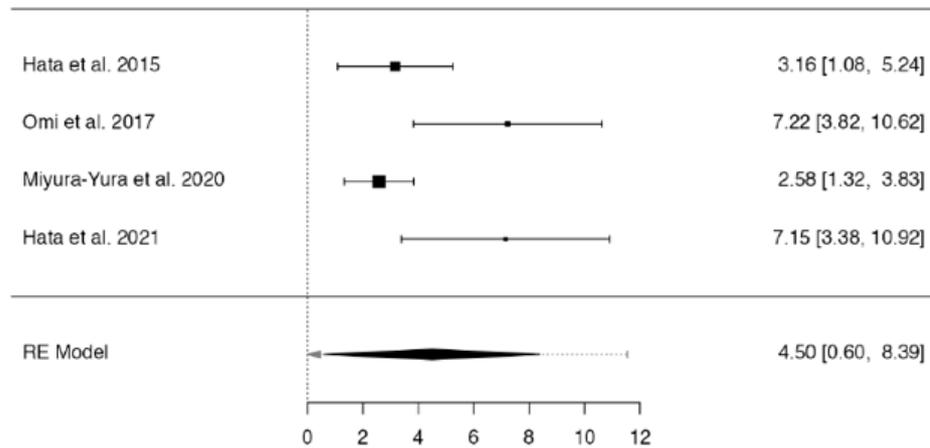
**Figure 2.** Effects of dental stem cells on blood glucose. (RE—random-effects, k—number of studies in analysis, SE—standard error, Z—test for overall effect, p—level of statistical significance, CI—confidence interval, Tau<sup>2</sup>—absolute value of variance i.e., heterogeneity among effect sizes, I<sup>2</sup>—statistic for degree of heterogeneity, df—degrees of freedom, Q—Cochran’s Q-test value).

There was substantial heterogeneity between studies (Q = 96.4187, p < 0.0001, tau<sup>2</sup> = 4.9123, I<sup>2</sup> = 88.5914%). A 95% prediction interval for true effects was estimated to be -9.0802 to 1.2702. Hence, the 95% range of true effects contained values that were less than zero, as well as values that were more than or equal to zero. This means that even though the average outcome (Z = -4.9737) and summary point estimate (SMD = -3.905) were negative, indicating a reduction in blood glucose, DSCs may in fact, have no effect or may even increase blood glucose in similar settings, with the greatest increase in blood glucose represented as SMD of 1.2702 in the analysis.

The studentized residuals showed that none of the studies could be considered to be outliers in the context of the analysis. In addition, none of the studies were overly influential according to Cook’s distances (data for studentized residuals and Cook’s distance analyses are available as supplementary data).

Effects of DSCs on SNCV

The SMDs ranged from 2.5757 to 7.2217 (Figure 3). The estimated average SMD was 4.4952 (95% CI: 0.5959 to 8.3945). The average outcome differed significantly from zero ( $Z = 3.6688, p = 0.0350$ ). Hence, following DSC administration, there was a statistically significant increase in SNCV compared to saline/vehicle.



Random-Effects Model (k = 4)

	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	4.50	1.23	3.67	0.035	0.596	8.394

Heterogeneity Statistics

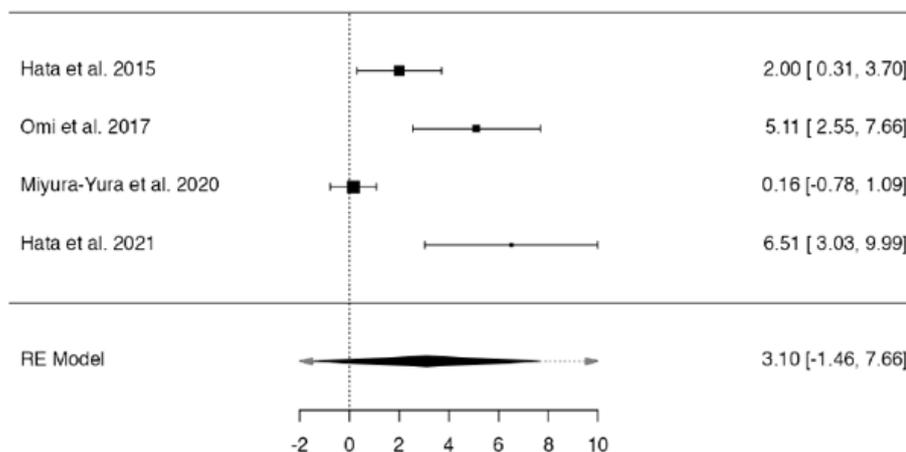
Tau	Tau <sup>2</sup>	I <sup>2</sup>	df	Q	p
1.843	3.3949 (SE= 4.3097)	71.08%	3.000	10.373	0.016

**Figure 3.** Effect of dental stem cells on sensory nerve conduction velocity (SNCV). (RE—random-effects, k—number of studies in analysis, SE—standard error, Z—test for overall effect, p—level of statistical significance, CI—confidence interval, Tau<sup>2</sup>—absolute value of variance i.e., heterogeneity among effect sizes, I<sup>2</sup>—statistic for degree of heterogeneity, df—degrees of freedom, Q—Cochran’s Q-test value).

The analysis revealed significant heterogeneity ( $Q = 10.3734, p = 0.0156, \tau^2 = 3.3949, I^2 = 71.0799\%$ ). A 95% prediction interval for true effects was estimated to be  $-2.5467$  to  $11.5371$ . Hence, although the average outcome ( $Z = 3.6688$ ) and summary point estimate (SMD = 4.4942) were positive, indicating an increase in SNCV after DSC administration, the SNCV may remain unchanged or may even decrease following DSC administration in a future study in a similar setting, with the greatest reduction in SNCV represented as SMD of  $-2.5467$ . The studentized residuals revealed that there were no outliers in the context of this model. According to Cook’s distances, none of the studies could be considered to be overly influential.

Effects of DSCs on MNCV

The SMDs of studies ranged from 0.1556 to 6.5147 (Figure 4). The average SMD was 3.1001 (95% CI: -1.4558 to 7.6559). The average outcome did not differ significantly from zero ( $Z = 2.1655, p = 0.119$ ). Hence, the increase in MNCV after DSC treatment compared to that following saline/vehicle administration was not significant.



Random-Effects Model (k = 4)

	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	3.10	1.43	2.17	0.119	-1.456	7.656

Heterogeneity Statistics

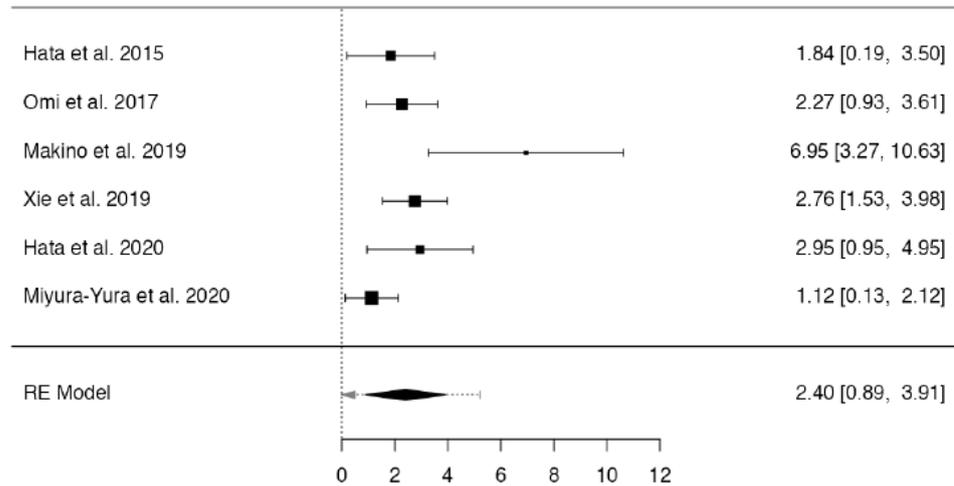
Tau	Tau <sup>2</sup>	I <sup>2</sup>	df	Q	p
2.464	6.0734 (SE= 6.665 )	87.28%	3.000	23.583	<.001

**Figure 4.** Effects of dental stem cells on motor nerve conduction velocity (MNCV). (RE—random-effects, k—number of studies in analysis, SE—standard error, Z—test for overall effect, p—level of statistical significance, CI—confidence interval, Tau<sup>2</sup>—absolute value of variance i.e., heterogeneity among effect sizes, I<sup>2</sup>—statistic for degree of heterogeneity, df—degrees of freedom, Q—Cochran’s Q-test value).

There appeared to be substantial heterogeneity ( $Q = 23.5832, p < 0.0001, \tau^2 = 6.0734, I^2 = 87.2791\%$ ). A 95% prediction interval for true effects was estimated to be -5.9701 to 12.1702. Hence, although the average outcome ( $Z = 2.1655$ ) and summary point estimate (SMD = 3.1001) were positive, indicating improvement in MNCV after DSC administration, the MNCV might remain unchanged or may even reduce following DSC administration in similar settings. In some cases, the result of DSC treatment may even be the exact opposite of the summary point estimate SMD, i.e., -3.1001 instead of 3.1001, with the greatest reduction in MNCV represented as SMD of -5.9701 in the analysis. The studentized residuals revealed that none of the studies could be considered as outliers in the analysis. According to Cook’s distances, none of the studies were overly influential.

Effects of DSCs on Capillary–Muscle Ratio

The SMDs of the studies ranged from 1.1249 to 6.9490 (Figure 5). The estimated average SMD was 2.4027 (95% CI: 0.8923 to 3.9132). The average outcome was significantly different from zero ( $Z = 4.0891, p = 0.0095$ ) (Figure 4). Hence, the analysis indicated that the capillary–muscle ratio increased significantly in favor of DSC treatment compared to saline/vehicle administration.



Random-Effects Model (k = 6)

	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	2.40	0.588	4.09	0.009	0.892	3.913

Heterogeneity Statistics

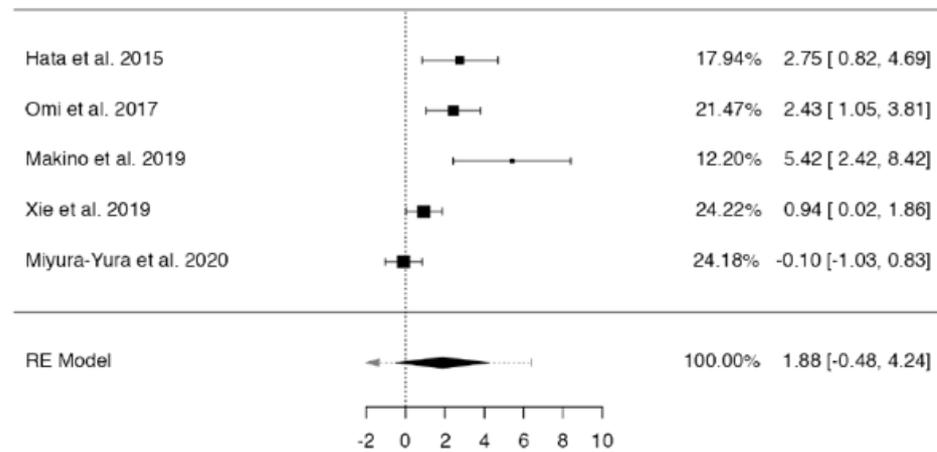
Tau	Tau <sup>2</sup>	I <sup>2</sup>	df	Q	p
0.920	0.8465 (SE= 0.9565 )	59.3%	5.000	12.284	0.031

**Figure 5.** Effects of dental stem cells on capillary–muscle ratio (RE—random-effects, k—number of studies in analysis, SE—standard error, Z—test for overall effect, p—level of statistical significance, CI—confidence interval, Tau<sup>2</sup>—absolute value of variance i.e., heterogeneity among effect sizes, I<sup>2</sup>—statistic for degree of heterogeneity, df—degrees of freedom, Q—Cochran’s Q-test value).

There appeared to be heterogeneity among studies ( $Q = 12.2844, p = 0.0311, \tau^2 = 0.8465, I^2 = 59.2979\%$ ). A 95% prediction interval for true effects was  $-0.4035$  to  $5.209$ . Hence, although the average outcome ( $Z = 4.0891$ ) and summary point estimate (SMD = 2.4027) were positive, indicating that capillary-muscle ratio improved with DSC administration, it may in fact remain unchanged or even reduce following DSC administration in similar settings, with the greatest reduction in capillary–muscle ratio represented as SMD of  $-0.4035$ . The studentized residuals revealed that none of the studies could be considered as outliers in the context of the analysis. According to Cook’s distances, none of the studies were overly influential.

Effects of DSCs on IENFD

The SMDs ranged from  $-0.0994$  to  $5.4206$  (Figure 6). The average SMD was calculated to be  $1.8802$  (95% CI:  $-0.4809$  to  $4.2413$ ). The average outcome did not differ significantly from zero ( $Z = 2.211$ ,  $p = 0.0915$ ). Hence, the increase in IENFD was not statistically significant following DSC administration compared to saline/vehicle.



Random-Effects Model (k = 5)

	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	1.88	0.850	2.21	0.092	-0.481	4.241

Tau	Tau <sup>2</sup>	I <sup>2</sup>
1.387	1.9243 (SE= 1.926 )	80.83%

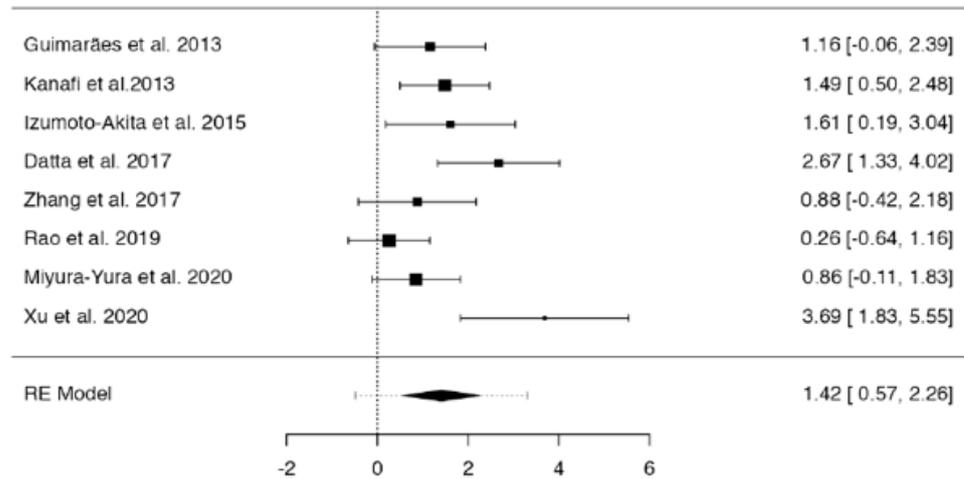
df	Q	p
4.000	20.862	<.001

**Figure 6.** Effects of dental stem cells on intra-epidermal nerve fiber density (IENFD). (RE—random-effects, k—number of studies in analysis, SE—standard error, Z—test for overall effect, p—level of statistical significance, CI—confidence interval, Tau<sup>2</sup>—absolute value of variance i.e., heterogeneity among effect sizes, I<sup>2</sup>—statistic for degree of heterogeneity, df—degrees of freedom, Q—Cochran’s Q-test value).

There appeared to be substantial heterogeneity ( $Q = 20.8617$ ,  $p = 0.0003$ ,  $\tau^2 = 1.9243$ ,  $I^2 = 80.8261\%$ ). A 95% prediction interval for true effects was estimated to be  $-2.6374$  to  $6.3978$ . Hence although the average outcome ( $Z = 2.211$ ) and summary point estimate (SMD =  $1.8802$ ) were positive, indicating an increase in IENFD after DSC administration, it may remain unchanged, or may even reduce following DSC administration in similar settings. In some cases, DSC treatment may even result in the exact opposite of the summary estimate, i.e.,  $-1.8802$  instead of  $1.8802$ , with the greatest possible reduction in IENFD represented as  $-2.6374$  in the analysis. The studentized residuals revealed that none of the studies could be considered outliers in the context of this model. According to Cook’s distances, none of the studies were overly influential in the analysis.

Effect of DSCs on Body Weight

The SMDs of the individual studies ranged from 0.2578 to 3.691 (Figure 7). The estimated average SMD was 1.415 (95% CI: 0.5674 to 2.2627). The average outcome differed significantly from zero ( $Z = 3.9475, p = 0.0056$ ). Hence, the increase in body weight with DSC administration was statistically significant compared to saline/vehicle.



Random-Effects Model (k = 8)

	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	1.42	0.358	3.95	0.006	0.567	2.263

Heterogeneity Statistics

Tau	Tau <sup>2</sup>	I <sup>2</sup>	df	Q	p
0.719	0.5169 (SE= 0.4818 )	58.86%	7.000	17.014	0.017

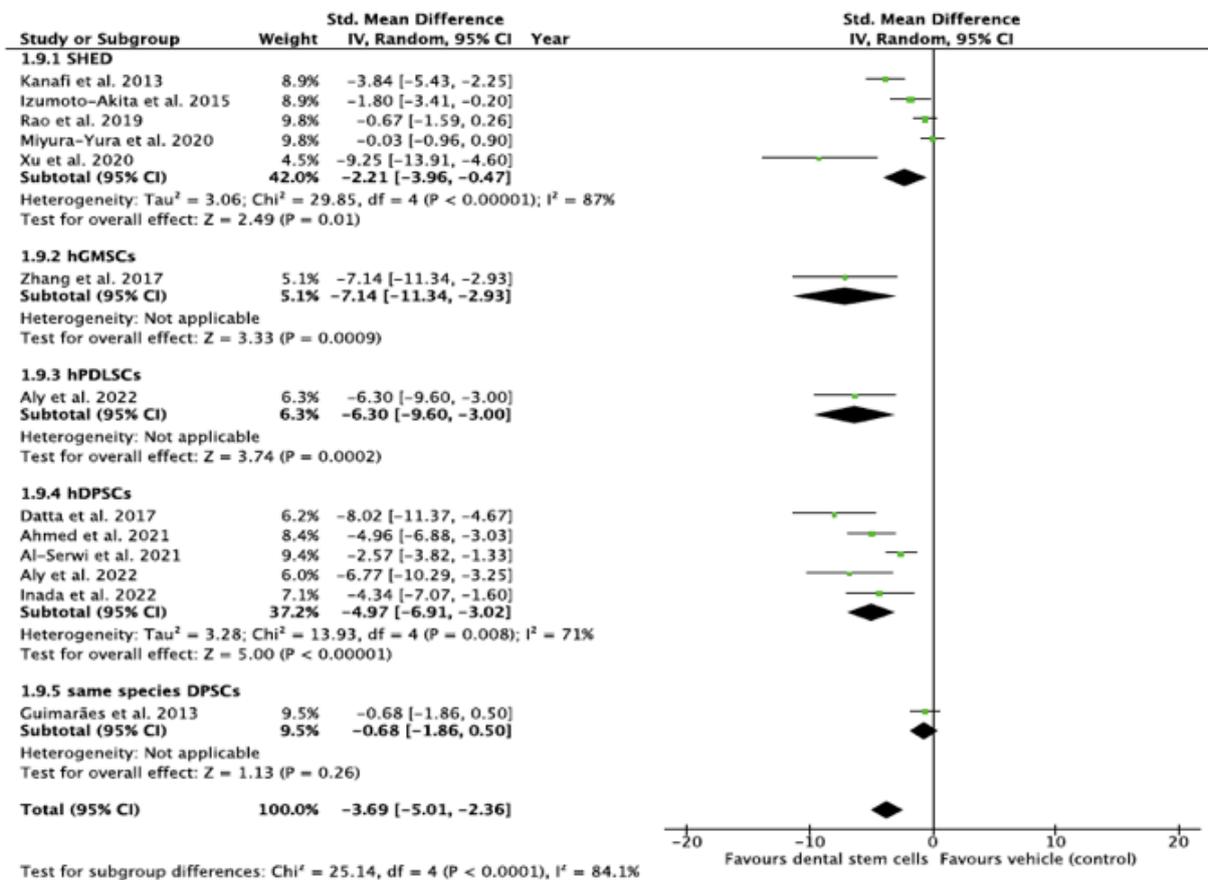
**Figure 7.** Effects of dental stem cells on body weight. (RE—random-effects, k—number of studies in analysis, SE—standard error, Z—test for overall effect, p—level of statistical significance, CI—confidence interval, Tau<sup>2</sup>—absolute value of variance i.e., heterogeneity among effect sizes, I<sup>2</sup>—statistic for degree of heterogeneity, df—degrees of freedom, Q—Cochran’s Q-test value).

There appeared to be heterogeneity ( $Q = 17.0143, p = 0.0173, \tau^2 = 0.5169, I^2 = 58.8583\%$ ). A 95% prediction interval for true effects was estimated to be  $-0.4846$  to  $3.3146$ . Hence, although the average outcome ( $Z = 3.9475$ ) and summary point estimate (SMD = 1.415) were positive, indicating that the body weight of the diabetic animals increased with DSC administration, it may not be affected, or may even decrease with DSC administration in some studies in similar settings, with the greatest decrease in body weight observed to be SMD of  $-0.4846$ . None of the studies could be considered to be outliers or to be overly influential, according to studentized residuals and Cook’s distances.

### 3.7.2. Subgroup Analysis

#### Subgroup Analysis of the Effect of DSC Type Used in the Study

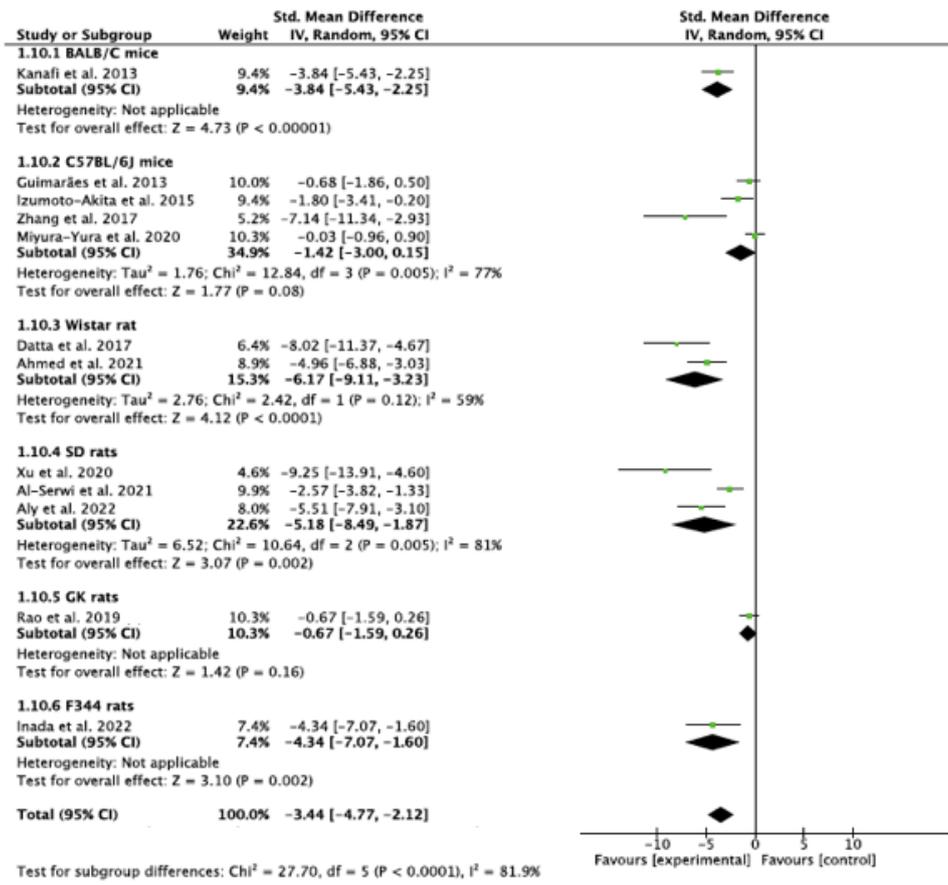
The test indicated that there is a statistically significant subgroup effect ( $p < 0.0001$ ), suggesting that DSC type may alter its effects on blood glucose (Figure 8). In all subgroups, DSCs were more beneficial in lowering blood glucose than saline/vehicle. Since the effect was seen more in some groups than in others, the subgroup effect was deemed quantitative. However, fewer studies were present in the hGMSCs, hPDLSC, and same species DPSC subgroups (Figure 8). In addition, there was substantial heterogeneity within the SHED ( $I^2 = 87%$ ) and hDPSC ( $I^2 = 71%$ ) subgroups. Due to the disparate number of studies and heterogeneity within the subgroups, the results of the analysis may not be reliable for interpretation.



**Figure 8.** Subgroup analysis with dental stem cell type as the variable. ( $Tau^2$ —value of variance among effect sizes,  $Chi^2$ —value of chi-squared test for heterogeneity,  $df$ —degrees of freedom,  $I^2$ —degree of heterogeneity,  $Z$ —test for overall effect,  $p$ —level of statistical significance).

#### Subgroup Analysis of the Effect of Species Variations

The analysis indicated that there is a statistically significant subgroup effect ( $p < 0.0001$ ), suggesting that differences in species may influence the effect of DSCs on blood glucose (Figure 9). In all subgroups i.e., among all strains of rodents used, DSCs were more beneficial in lowering blood glucose than saline/vehicle. Since the effect was seen more in some groups than in others, the subgroup effect is quantitative. However, there was disparity in the number of studies in subgroups. In addition, there was substantial heterogeneity within the C57BL/6 ( $I^2 = 77%$ ), SD ( $I^2 = 81%$ ), and Wistar rat ( $I^2 = 59%$ ) subgroups. Hence, the findings may not be considered reliable.



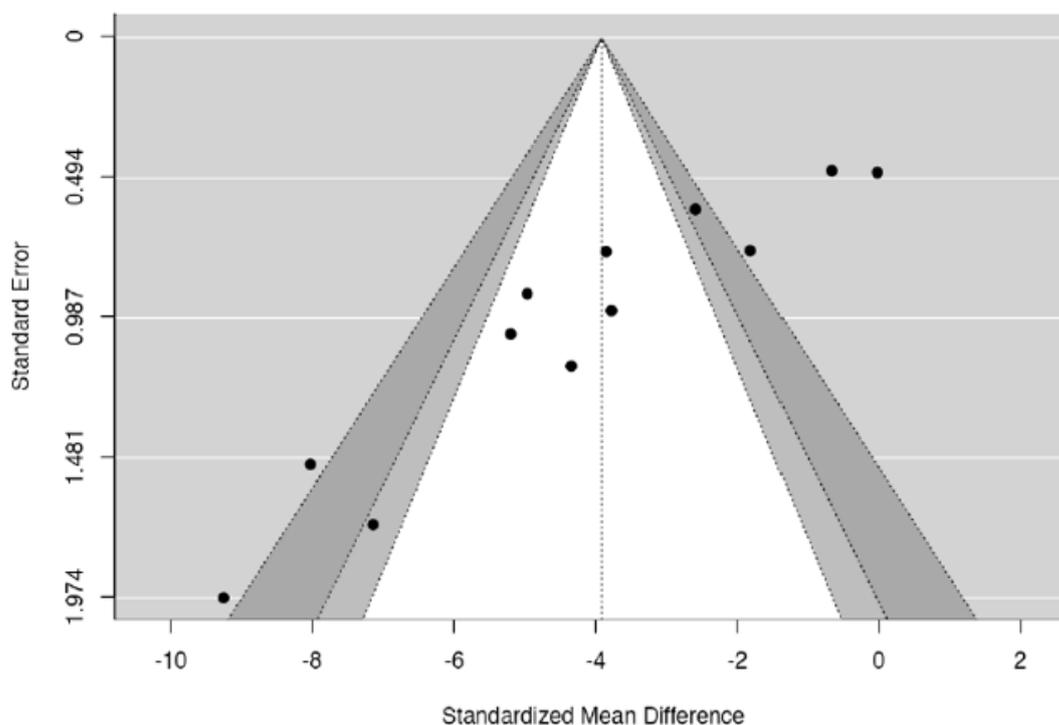
**Figure 9.** Subgroup analysis with species as the variable. (Tau<sup>2</sup>—value of variance among effect sizes, Chi<sup>2</sup>—value of chi-squared test for heterogeneity, df—degrees of freedom, I<sup>2</sup>—degree of heterogeneity, Z—test for overall effect, p—level of statistical significance).

### 3.7.3. Funnel Plot Analysis and Publication Bias

Upon visual assessment, the funnel plot for DSC effects on blood glucose appeared asymmetrical around the vertical line representing the summary effect (Figure 10). Both regression and rank correlation tests indicated the presence of funnel plot asymmetry (p < 0.0001 and p = 0.0018, respectively) (Table 3). The asymmetry could be present due to publication bias, heterogeneity among the studies, methodological design of the studies, or chance [28,40].

**Table 3.** Egger’s regression and Begg and Mazumdar rank correlation tests for funnel plot.

Test	Value	p
Egger’s regression	-7.831	p < 0.0001
Begg and Mazumdar rank correlation	-0.667	p = 0.0018



**Figure 10.** Funnel plot.

#### 4. Discussion

To our knowledge, this is the first systematic review and meta analysis that explicates the scope of various dental stem cells in the management of hyperglycemia and DPN. Quantitative analysis in the present review indicated that there was a significant reduction in blood glucose ( $p = 0.0004$ ) in diabetes-induced animals following DSC administration.

The underlying mechanisms of DSCs have not been fully established. Following administration, they induce differentiation of pancreatic progenitor cells [59], promote transdifferentiation of  $\alpha$  into  $\beta$  cells [57], and modulate  $\beta$  cell function [55,57]. Emerging evidence indicates that extracellular apoptotic vesicles (apoVs) containing proteomes are pivotal in mediating MSC functions [70]. DSCs act by paracrine signaling, secreting VEGF and bFGF, which are important for tissue growth and regeneration [13,46,53]. They also release neurotrophin-3 (NT-3) and NGF, which are essential in neuronal development and repair [13,49,50,53].

The pooled effects showed that SNCV ( $p = 0.035$ ) and capillary–muscle ratio ( $p = 0.0095$ ) improved significantly following DSC administration, but IEFND ( $p = 0.0915$ ) and MNCV ( $p = 0.119$ ) did not. IEFND is used to measure neuropathy of small fibers, such as unmyelinated C and A $\delta$  fibers. The findings are consistent with a study in which, 16 weeks after T1DM was induced and 4 weeks after SHED-CM was administered, there was neither any improvement in IEFND, nor in thermal and tactile sensitivity tests, indicating that DSCs were unable to reverse advanced stages of hypoalgesia in DM [50]. In addition, due to the larger diameter of motor nerves, changes in MNCV occur later than those in SNCV [50]. In the same study, 4 weeks after SHED-CM was administered, MNCV did not improve, suggesting that the duration of the experiment may have been insufficient to detect changes in MNCV [50]. Hence, the review reflects that studies with longer follow-up periods, using long-term diabetes-induced animals, may be most appropriate to demonstrate the effects of DSCs on IEFND and MNCV in advanced stages of DPN.

Human derived DPSCs were able to transdifferentiate into insulin-producing  $\beta$  cells in the murine pancreas following IV and intrapancreatic administration [59]. Interestingly, the new cells were morphologically identical to those of the recipient animals, and the levels of human, as well as murine insulin increased in these animals [59].

A reduction in TNF- $\alpha$  and an increase in IL10 [47] was observed in the DSC-administered muscle on one side, compared to the contralateral side of the same animal. This is connotative of the local effects of DSCs. On the other hand, IV administration also resulted in significant improvement in hyperalgesia and late onset hypoalgesia, indicating a systemic effect by reducing blood glucose [63]. DSC engraftment in injured organs and the subsequent amelioration of DPN [63], liver disease [56], and impaired salivary flow [58] following IV administration may be suggestive of both local and systemic effects.

The IV route of DSC administration is congruent with MSC-based therapy for controlling hyperglycemia and systemic inflammation [14]. On the other hand, a single IM dose of hDPSCs resulted in improvement in DPN parameters which lasted for 16 weeks in T1DM induced rats [48]. Irrespective of the route, repeat doses may be more effective in long-term diabetes due to sustained effects on cytokines [17,55,63]. Li et al. (2021) reported that the effective rate of three IV doses of SHED, at the end of a 12 month follow up for diabetic patients was 68.18%, suggesting that therapy may be effective for at least one year [6].

The viability of SCs is affected by aging and disease; hence, it is important to isolate them from healthy teeth at a young age [13,46,48]. Cryopreservation may hold the key to effective autologous DSC therapy, as they can be isolated from healthy teeth which have been extracted at a young age, thawed, and expanded in culture when needed [13,46,48]. Cryopreserved rDPSCs demonstrated proliferative capacities that were comparable to freshly isolated rDPSCs in T1DM rats, and were able to maintain their viability for at least 6 months before administration [46]. However, cryopreservation should be studied further before clinical application with DSCs due to the risk of solution effect injury, toxicity, arrhythmia, and hypotension [71].

#### 4.1. Scope of Translation of Dental Stem Cell-Based Therapy in Diabetes Mellitus

For research to be effectively translated, studies should accurately predict the clinical course in humans. In the presence of substantial heterogeneity among studies, the prediction interval in each analysis demonstrates how different the true effect in a new study might be from those observed in previous similar studies. It thus, also provides insight into the uncertainty in predicting the effect in settings that may be different from those included in the analysis, thereby pointing to the challenges in translation to clinical settings.

It was unclear whether adequate randomization, allocation, concealment, and blinding were followed in the animal studies included in the review (Table 2). Failure to adhere to these principles may have led to overestimating the efficacy of DSCs.

In order to optimize their contribution to clinical practice, studies should reflect the disease as well as the population for which the intervention is intended. The homogeneity in animal samples may render them unrepresentative of heterogeneous human populations [65,66]. Further, the selection of healthy animals with no prior disease, as well as shorter duration of most of the studies, do not reflect the complexity of diabetes, nor recapitulate its slow and progressive nature [65,66]. The timing of the intervention should also model the delay between the onset of symptoms and treatment that often occurs in humans. Another factor is that many organ systems are still developing in the age range of the animals used in the studies, and the changes that occur in this phase could have affected the variables that were being measured [67,68]. Moreover, diabetes, particularly T2DM, typically manifests in older individuals. In addition, although sex effects are present in diabetes, it is widely prevalent in both males and in females [72], whereas only one animal study demonstrated the effects of DSCs in female mice [63]. These factors may impede clinical translation.

The most intractable aspect of animal studies undermining their external validity is the inherent interspecies differences [65,66]. Rodents are used in biomedical research due to their genetic and physiological similarities to humans [12]. Their life stages mimic those in humans [12]. However, modern rodents have adapted to their own unique environment and have evolved to respond to diseases and interventions differently than humans [65]. Moreover, genetic variations exist, even within individual strains of the same species, such

as C57BL/6 mice and GK rats, which must be considered while designing a study and interpreting its results [12].

Similar to the animal studies, the follow-up phase in the clinical study was adequate to monitor immediate and early adverse effects, but may not have been sufficient to determine long-term complications. Sometimes, certain properties of a drug may go unnoticed, even after it has passed safety checks in preclinical and short-term clinical studies. An example is the antiviral drug fialuridine, which had potential use in the treatment of hepatitis B [73]. It had passed preclinical investigations, and pilot studies with 2 and 4 week courses of the drug. However, during the 13th week of a phase 2 clinical trial, following administration of doses which were several hundred times smaller than the dose deemed safe in laboratory animals, patients suffered severe hepatotoxicity, resulting in the deaths of five patients [73]. The lesson to be learned from this tragedy is that not only are species variations underestimated, but that the duration of studies is also often insufficient to assess the risk of chronic toxicity. Even if a drug is deemed safe in animals at much higher doses, it may exhibit vastly different pharmacological properties in humans.

#### 4.2. Publication Bias

Both Egger's regression ( $p < 0.0001$ ) and Begg and Majumdar rank correlation ( $p = 0.0018$ ) tests indicated funnel plot asymmetry. One of the reasons for this asymmetry may be publication bias. This means that studies which reported amelioration of hyperglycemia were more likely to be published than studies that reported no change, or reported an increase in blood glucose. Such selective publication can hinder effective translation because the interpretation and implementation of data will be based only on partial evidence [66]. It results in the wastage of animals and resources used in unpublished studies.

#### 4.3. Graft Rejection, Tumorigenesis, and Other Adverse Reactions

MSCs are not completely immunoprivileged and sometimes undergo graft rejection [5]. Although all the studies including the clinical study [6], used allogeneic or xenogeneic sources for DSCs, only one animal study [54] described the use of tacrolimus. Most MSCs become trapped in the lungs at some point [5,6,57] or undergo apoptosis after administration [6]. However, their fate is still obscure. Engraftment occurred in organs such as the pancreas, liver, kidneys, and muscles following various routes of administration [9,14,46,48,55,58,63]; however, in one study, it was reported that after intraperitoneal administration, very few DSCs engrafted in the lungs and brain [9]. Hypoxia, hyperglycemia, and inflammation may contribute to the homing tendencies of MSCs [55]. In diabetes, there may be multiple organs with varying degrees of inflammation [74]. Hence, potential interactions in the local environment and the risk for tumorigenicity in every organ and tissue should be studied. Another factor to consider is the risk of teratoma, a phenomenon observed when embryonic stem cells are injected in mice, and a hallmark of pluripotent stem cells [75]. Although isolated DSCs are specifically induced in the laboratory to become committed to the desired cell phenotype *in vivo*, their broad spectrum differentiation potential must not be ignored. Common side effects of MSC therapy in DM, such as hypoglycemia, headache, fever, and rash, should also be considered [6].

Animal studies provide the foundation for testing new therapies and help to increase our understanding of an intervention. The present systematic review aimed to provide insight into the applicability of teeth and supporting tissues as potential sources of stem cells in the treatment of diabetes mellitus. The review also underscores the importance of methodological quality of animal studies to reliably inform clinical translation. In addition, the reviewers hope to contribute to implementing the 3 Rs, i.e., replacement, refinement, and reduction, by encouraging transparent reporting, responsible use of animals, and preventing replication of flawed study designs. The review has some limitations. Substantial heterogeneity was observed, which could be attributed to factors such as differences in species, the type and preparation of DSCs, the duration of the experiments, study design, and statistical differences in results between studies [28,40]. Meta-analysis was nevertheless

performed to provide quantitative assessment of the effects of DSCs and to demonstrate the differences in effects among studies. Furthermore, the disparate number of studies in the subgroups reduces the reliability of the subgroup analyses. Nonetheless, the analyses were included in the review due to the putative effects of SC type and species differences on experiment outcomes [5,8,12]. Lastly, the most crucial caveat for translation to humans is that all preclinical data should be interpreted with caution due to the irrevocable issue of interspecies differences.

## 5. Conclusions

The prospect of rapid and enduring glycemic control is exciting, as conventional drugs do not have lasting effects [63]. Within the limitations of this review, DSCs appear to be beneficial for glycemic control; however, the potential risk of graft rejection and tumorigenesis must not be ignored. It behooves researchers performing animal studies to adopt standards similar to those used in clinical trials, while considering methodological design and reporting. Studies with longer follow-up periods and greater sample power should be undertaken to determine long-term effects and track the fate of DSCs.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijtm4010005/s1>, Table S1: PRISMA 2020 Abstract checklist; Table S2: PRISMA 2020 checklist; Table S3: Full version of search filters; Table S4: Comparative analysis of included studies to determine external validity; Figure S1: Outliers and residuals for the analyses.

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