Universidad Europea VALENCIA

Grado en ODONTOLOGÍA

Trabajo Fin de Grado

Curso 2022-23

THERAPEUTIC POTENTIAL OF HUMAN DENTAL PULP STEM CELLS IN CARDIAC REPAIR, A NEW HOPE FOR INTERDISCIPLINARY REGENERATIVE MEDICINE? – A SYSTEMATIC REVIEW

Presentado por: Emilie Patricia Stéphanie CAULET

Tutor: Dra. María del Carmen CARCELLER ZAZO, PhD

Campus de Valencia
Paseo de la Alameda, 7
46010 Valencia
universidadeuropea.com

To my sister Mathilde Caulet,

Receive my deepest gratitude for your unwavering support and steadfast presence throughout this whole journey. Your vigilant guardianship and unconditional commitment have been invaluable to me, I am forever indebted to your loving devotion, and I will forever hold immense admiration for your strength. I humbly extend my sincere thanks to you and dedicate you this work.

To my parents Bernard and Magali Caulet,

I am deeply grateful for your continuous faith in my abilities. Your love, encouragement, and guidance has always been a solid pilar in my life. I am beyond grateful to have you and incredibly proud to be your daughter.

In loving memory of my grandfathers Doctor Jean-Louis Caulet and Maurice Giordanengo,

It is with profound reverence that I pay tribute to these outstanding men, whose never ending determination and courage have consistently been inspiring me, urging me to approach every endeavor with passion, dedication, patience and resilience. Their legacies embody the very essence of devotion and excellence. May they receive through this work my respectful tribute.

To my grandmothers, the late Monique Caulet and Monique Giordanengo,

I wish to honor the dedication and perseverance of these two remarkable women, whose commitment to their families lighted the way throughout my journey.

To my tutor Doctor María del Carmen Carceller Zazo who directed this work,

Through your trust and expertise, you granted me the freedom to realize exactly what I envisioned for this work. You allowed me to build bridges between medicine and dentistry. For this, Receive the formal expression of my sincere gratitude. Your precious guidance and direction lead me through the complexity of this work.

To my professor Doctor Patricia Guillem Saiz, patron of our graduation class,

For your generous efforts, and commitment. Your continuous support and presence have influenced my academic and personal growth, thank you for your precious proficiency.

To all those who have ever experienced failure and found the strength to never give up and pursue their dreams.

TABLE OF CONTENT

Abbreviations

BMMSC	Bone marrow-derived mesenchymal stem cells
COVID-19	Coronavirus disease 2019
DMSO	Dimethyl sulfoxide
DPSCs	Human dental pulp stem cells
EGF	Epidermal growth factor
FS	Fractional shortening
hBMMSC	Human bone marrow-derived mesenchymal stem cells
H&E	Hematoxylin & Eosin
LVDd	Left Ventricular End-Diastolic dimension
LVDs	Left Ventricular End-Systolic dimension
MI	Myocardial infarction
MSCs	Mesenchymal stem cells
SDF-1	Stromal cell-derived factor
SHED	Dental pulp stem cells isolated from the pulp of human exfoliated deciduous teeth
TGF-ß	Transforming growth factor ß
VEGF	Vascular endothelial growth factor

1. ABSTRACT

Introduction: Over the past 2 decades, mesenchymal stem cells (MSCs) have become the basis of the emerging field of regenerative medicine due to their self-renewal and multilineage differentiation abilities. Despite treatment advancements, myocardial infarction remains a significant cause of mortality and disabilities. Dental pulp stem cells (DPSCs) offer promising regenerative properties for cardiac regeneration. This review aims to assess the improvement achieved by human DPSCs and stem cells from human exfoliated deciduous teeth (SHED) in cardiac function, infarct size reduction, and angiogenesis compared to human bone marrow MSCs (hBMMSC) in rodent models of myocardial infarction.

Material and Methods: An electronic search was conducted in PubMed, Scopus and Web of Science databases on cardiac regeneration achieved by DPSCs, SHED or hBMMSC in rodent models of myocardial infarction. The search was completed by manual search. The limitation date was January 2023.

Results: Above 844 studies identified through the search process, 6 studies were included for complying with the inclusion criteria: 1 study describes the transplantation of SHED, 4 describe the transplantation of hBMMSC, and 1 describes the transplantation of DPSCs. Cardiac regeneration was evaluated through 3 parameters: Cardiac function improvement (through fractional shortening (FS) evolution), infarct size reduction and angiogenesis. Regarding FS improvement: DPSCs showed a mean increase of 36.95%, hBMMSC 27.13%, and SHED did not provide any significant result. Infarct size was reduced by 25% with DPSCs, 45.2% with SHED and 18.67% with hBMMSC. Angiogenesis was observed in all groups but not quantified in the SHED group, DPSCs group presented a mean increase of vascular density of 67.51% and the hBMMSC group 34.57%.

Conclusion: Despite limitations, dental pulp stem cells seemed to achieve greater improvement in cardiac function, reduction of the infarct size as well as induction of angiogenesis compared to hBMMSC in rodent models of myocardial infarction.

2. RESUMEN

Introducción: En las últimas 2 décadas, las células madre mesenquimales (MSCs) se han convertido en la base del campo emergente de la medicina regenerativa debido a su capacidad de autorrenovación y diferenciación multilinaje. A pesar de los avances en el tratamiento, el infarto de miocardio sigue siendo una causa importante de mortalidad y discapacidad. Las células madre de la pulpa dental (DPSCs) ofrecen prometedoras propiedades regenerativas para la regeneración cardiaca. El objetivo de esta revisión es evaluar los efectos de las DPSCs humanas y las células madre de dientes temporales exfoliados humanos (SHED) en la función cardiaca, la reducción del tamaño del infarto y la angiogénesis en comparación con las MSCs de médula ósea humana (hBMMSC) en modelos de infarto de miocardio en roedores.

Material y métodos: Se ha realizado una búsqueda electrónica en las bases de datos PubMed, Scopus y Web of Science sobre la regeneración cardiaca lograda por DPSCs, SHED o hBMMSC en modelos de infarto de miocardio en roedores. La búsqueda se completó mediante búsqueda manual. La fecha límite fue enero de 2023.

Resultados: De los 844 estudios identificados mediante el proceso de búsqueda, se han incluido 6 estudios por cumplir los criterios de inclusión: 1 estudio describe el trasplante de SHED, 4 describen el trasplante de hBMMSC, y 1 describe el trasplante de DPSCs. La regeneración cardiaca se ha evaluado a través de 3 parámetros: mejora de la función cardiaca (a través de la evolución de la fracción de acortamiento (FS)), reducción del tamaño del infarto y angiogénesis. En cuanto a la FS las DPSCs mostraron un aumento medio del 36,95%, las hBMMSC del 27,13%, y el grupo con SHED no proporcionó ningún resultado significativo. El tamaño del infarto se redujo en un 25% con DPSCs, un 45,2% con SHED y un 18,67% con hBMMSC. La angiogénesis se observó en todos los grupos, pero no se cuantificó en el grupo SHED, el grupo DPSCs presentó un aumento medio de la densidad vascular del 67,51% y el grupo hBMMSC del 34,57%.

Conclusiones: A pesar de las limitaciones, las células madre de la pulpa dental parecen conseguir una mayor mejora de la función cardiaca, una mejor reducción del tamaño del infarto, así como una mejor inducción de la angiogénesis en comparación con las hBMMSC en los modelos de infarto de miocardio en roedores.

3. KEYWORDS

I. Myocardial infarction
II. Rodents
III. Human dental pulp stem cells
IV. Dental pulp stem cells isolated from the pulp of human exfoliated deciduous teeth
V. Human bone marrow-derived mesenchymal stem cells
VI. Stem cell therapy
VII. Cardiac repair
VIII. Cardiac function improvement
IX. Infract size
X. Ventricular function
XI. Angiogenesis

4. INTRODUCTION

4.1 Generalities

In a world of constant evolution dentistry has recently been embossing its presence through major leaps in interdisciplinary medical research. Dental professionals are consistently seeking to improve patient treatment.

Over the past 2 decades, mesenchymal stem cells (MSCs) have become the basis of the emerging field of regenerative medicine thanks to their abilities of self-renewal and multilineage differentiation (1). They are to be found in almost all tissues such as teeth, adipose tissue, muscle, umbilical cord blood, peripheral blood, liver, placenta, skin, amniotic fluid, breast milk and synovial membrane (2–4). Although the regenerative capacity of human dental tissue is limited, the discovery of permanent dentition dental pulp stem cells (DPSCs) as well as dental pulp stem cells isolated from the pulp of human exfoliated deciduous teeth (SHED) in the early 2000s, opened a new and surprising outline in tooth regeneration as well as non-dental tissue regeneration leading the way to interdisciplinary collaboration (5–7).

Above all adults' stem cells, dental stem cells are the most accessible. They are available from the pulp of both permanent and temporary dentition, the dental follicle (mesenchymal cells and fibers surrounding the developing tooth), the periodontal ligament (soft tissue union between the tooth and the bone), and tips of developing roots (7).

While present studies are mostly conducted with human bone marrow-derived mesenchymal stem cells (hBMMSC), DPSCs and SHED showed a higher proliferation rate compared to hBMMSC as well as a differentiating potential into myocytes, melanocytes, active neurons, hepatic-like cells, insulin-producing cells and cardiomyocytes (8–16). Stem cell therapy is already common in the medical field for: bone defects, Alzheimer's disease, diabetes mellitus, spinal cord injuries, degenerative diseases, and myocardial infarction (MI) (17).

Despite recent substantial advances in prevention and treatment, MI and cardiovascular diseases remain a leading cause of death with a prevalence of 3 million people worldwide annually causing irreversible damage to cardiomyocytes due to oxygen supply discontinuation (18). The lack of regenerative capacity of the myocardium highlights the importance of reducing cardiomyocyte loss, stimulating neovascularization, and maintaining cardiac function. Thus, dental stem cell transplantation appears to be a solid option in the field of regenerative medicine and currently delivers promising results in cardiac regeneration.

In this systematic review we aim to compare the therapeutic potential of human dental pulp stem cells (DPSCs & SHED) versus hBMMSC in myocardial repair after myocardial infarction in rodents.

4.2 hBMMSC

hBMMSC -discovered in the 1970s by A.J. Friedenstein-, originate from the mesoderm. They are the multipotent adult nonhematopoietic cells that established the idea of using either allogeneic or autologous stem cells to repair damaged tissues, by regenerating and rejuvenating permanently damaged organs (19).

hBMMSC are to this day the most studied candidate for cardiovascular repair and are thought to be one of the most promising treatments thanks to their multipotency and ability to differentiate into: smooth cells, fibroblasts, cardiomyocytes, and endothelial cells. Despite their ability to differentiate into cardiomyocytes and to express troponin T and GATA4 (both markers of cardiac lineage), they demonstrated *in vitro* to lack sarcomeric organization (20).

Easily accessible, but to be found in limited quantities, hBMMSC can be obtained from bone marrow biopsy and can be used for autogenic graft or allogenic transplant between compatible patients (19).

Interestingly, while maxillary and mandibular bones are derived exclusively from cranial neural crest cells, the iliac crest bone is formed by mesoderm. These differences in embryological origins may result in functional differences between human orofacial and iliac crest hBMMSC used for regenerating purposes (21).

4.3 Dental stem cells

The dental pulp is a loose connective tissue occupying a cavity called the pulp chamber, this chamber is surrounded by a calcified tissue called dentin. Dental pulp contains immune cells such as: macrophages, lymphocytes, and dendritic cells. It is also formed by odontoblasts and fibroblasts respectively in charge of forming dentin and producing collagen. Finally, the dental pulp is made of stem cells known as DPSCs in adult dentition and SHED in deciduous dentition.

Extracted teeth such as bicuspids for orthodontic purposes, extracted third molars, or extracted deciduous teeth constitute an easily obtainable source of dental stem cells making dental pulp a great source of post-natal stem cells readily available with minimally invasive technique and minimum trauma (22).

4.3.1 DPSCs

DPSCs originate from cranial neural crest ectomesenchyme, are multipotent, and are to be found in adult permanent dentition pulp. DPSCs express the surface markers CD73, CD90, and CD105 but lack the expression of CD34, CD14, CD45, and human leukocyte antigen (HLA)-D, thus they fulfill the requirements to be considered as mesenchymal stem cells (6).

DPSCs are in control of the dentin-pulp repairing complex maintaining homeostasis after an exogenous traumatic stimulus such as caries for example. This regenerative capacity of the human dentin/pulp complex led Gronthos *et al.* (5) to believe and demonstrate that dental pulp contains progenitors that are responsible for dentin repair.

Hence, depending on the signaling received, DPSCs possess the ability to regenerate new stem cells or undergo a differentiation process. Armiñán *et al.* (23) demonstrated, for example, the differentiation potential of DPSCs into cells with cardiac phenotype during co-culture with neonatal rat cardiomyocytes.

4.3.2 SHED

Derived from the cranial neural crest ectomesenchyme, SHED are multipotent and to be found in the immature deciduous pulp.

In vitro, SHED presented higher proliferative and clonogenic immature multipotent properties than DPSCs and BMMSCs, (SHED > DPSCs >BMMSCs) (24–26).

Around 4386 genes were found to have a two-fold or greater difference in expression between DPSCs and SHED when the gene expression profiles were compared. Genes involved in extracellular matrix production and cell proliferation, including many growth factors such as fibroblast growth factor and transforming growth factor (TGF)-ß, were found to have higher expression in SHED (16).

Kerkis *et al.* (27) demonstrated that SHED possess myogenic potential. Nevertheless, autologous stem cell therapy with infant stem cells for a disease such as myocardial infarction would require for these cells to be stored from childhood until the appearance of the disease, thus introducing the concept of stem cell banking.

4.4 Dental stem cells banking

In an era where extracted teeth such as deciduous teeth or adult 3rd molars are most likely to be considered biological trash, the prospect of using dental stem cells for autologous transplant, particularly in the case of neurodegenerative diseases and cardiovascular diseases gives rise to the concept of dental stem cell banking.

The concept of stem cells banking involves the harvesting and storage of dental stem cells with the aim of being used for regenerative needs in the future. The process can be summed up into 3 main parts: tooth collection, isolation of stem cells and preservation (17,28–30)

The best candidates for pulp isolation in deciduous dentition are canines and incisors. Regarding permanent dentition: premolars and third molars are also considered good candidates for cell banking (24).

4.4.1 Tooth collection

Freshly extracted teeth will be placed in a sterile saline solution with frozen gel packs. They are then transferred in a vial containing hypotonic phosphate buffered saline solution which will help to preserve vital tissues during transport.

The package that will be sent to the laboratory must be sealed and placed into a temperature phase change carrier which will then be placed into an insulated metal transport vessel (29).

4.4.2 Stem cell isolation

Pulp will be isolated and cultured in a MSCs medium under appropriate conditions which will condition their lineage (29).

4.4.3 Preservation

Preservation can be achieved through 2 main processes: Cryopreservation and Magnetic freezing (29).

Considering recent major advancements in cryopreservation technology, hematopoietic stem cells remain to be the most cryopreserved cells that can be effectively used for transplantation. The main challenge of long-term preservation resides in potential

deleterious effects such as senescence, cell death, contamination, or phenotypic instability (31).

Conventionally, the process of cryopreservation involves disinfection of the tooth, pulp extraction, cell isolation, proliferation, and finally cryopreservation (28).

The rate of cooling is one of the most crucial factors in the cell freezing process. Osmotic stress causes cells to dry and shrink when they are cooled too slowly, yet rapid cooling causes the production of intracellular ice which causes cell death. DPSCs monolayers were found to maintain membrane integrity when subjected to 100% intracellular ice production, however they lost their capacity to proliferate (32)

Cell membrane protection can be increased by a dimethyl sulfoxide (DMSO) solution, able to penetrate the cell membrane and thus protect it from rupture. It was proven that optimal cryopreservation protocols were the ones conducted at -1°C/min in an isopropanol bath to -85°C with the addition of a solution of 7,8-11,6% of DMSO followed by storage in nitrogen. After thawing, DPSCs maintained their stem cell markers as well as their capacity for multilineage differentiation (30).

Regardless of the extensive use of DMSO, it is believed to have cytotoxic effects involving defects after transplantation (33). Facing this, research focused on xeno-free conditions which showed an effectivity of 510 days free of karyotype abnormalities and stable division times comparable to those of fresh DPSCs (34).

SHED, showed that they do maintain their properties after cryopreservation for 2 years (35).

Magnetic freezing also known as “Cell Alive System”, is based on the use of a magnetic field chilling cells below freezing point ensuring distributed low temperature that is believed to not damage the cell wall. Hiroshima University claimed that this technique can increase the cell survival rate by up to 83% (29).

4.5 Myocardial infarction

4.5.1 Pathogenesis

MI occurs when there is an interruption of blood flow in coronary arteries due to cholesterol and fat deposits resulting in blood clots, depriving the myocardium of oxygen and thus causing muscle necrosis because of lack of oxygen. Ischemic heart disease is usually implying healed infarct, foci of myocardial scarring, cavitary dilation, and impaired ventricular performance. Symptoms go from chest pain that extends to the left arm or left side of the neck, nausea, shortness of breath, abnormal heart rate, anxiety, fatigue, sweating, and vomiting to a variety of other less specific symptoms (36).

The most immediate way to pharmacologically act to reduce complications is the intake of acetylsalicylic acid (to prevent blood clots) and nitroglycerin (to provoke vasodilatation) (37).

During the first 30 minutes of ischemia, structural changes can be observed in cardiomyocytes as well as a developing oedema generating progressive death of cardiac cells. Secondly, reperfusion itself will be responsible for a second phase of injury with the production of reactive oxygen species. The embolization of thrombotic debris after reperfusion will give rise to microvascular dysfunction and finally provoke heart failure.

As a consequence of cardiomyocyte death and scar formation, a chronic neurohumoral activation (renin–angiotensin–aldosterone and sympathetic nervous system up-regulation), as well as ventricular remodeling, will take place. Ventricular remodeling is defined by a change in ventricular geometry leading to wall thinning, ischemic mitral regurgitation, and even more cardiomyocyte loss (38).

Interestingly in the actual sanitary context of the coronavirus disease 2019 (COVID-19) pandemic, it has been observed an existing potential higher risk of heart failure development after MI in patients infected with COVID-19. There is also growing evidence of direct myocardial injuries such as troponin elevation in patients infected

with COVID-19. Relations between COVID-19 and cardiovascular defects are still under investigation (38,39).

4.5.2 Cardiac function assessment

Real time images of the heart can be obtained through echocardiography. This sound waves based non-invasive technique allows us to evaluate cardiac anatomy function (40,41). Above all measurement used to evaluate cardiac function measurement: Left Ventricular End-Diastolic dimension (LVDd), Left Ventricular End-Systolic dimension (LVDs) and Fractional shortening (FS) were the ones used in this systematic review to quantify how well left ventricle is able to pump blood at each heartbeat.

Myocardial infarct will have consequences on left ventricle function and anatomy. LVDd can increase due to the loss of functionality of cardiac cells, this increase will trigger a diminution of heart's contractility and a decrease in FS (40–43).

4.5.3 Potential mechanisms of regeneration and therapeutic outcomes

The human heart lacks any regenerative capacity. Current research is focused on the use of hematopoietic stem cells as well as the use of mesenchymal stem cells for cardiac repair.

Regarding hematopoietic stem cells and cardiac therapy, research demonstrated that despite of their potent proangiogenic power, they exhibited a limited ability to provide proper regenerative treatment (44). Investigating the mechanisms by which mesenchymal dental stem cells could potentially regenerate infarcted heart tissue is of utter importance for the development and improvement of new therapeutic leads.

Mesenchymal stem cell therapy and heart regeneration are mainly based on indirect (paracrine signaling) and direct (trans-differentiation) mechanisms as well as neovascularization, immunomodulation, and cardiac remodeling (45–48).

Transdifferentiation mechanism involves a direct cardiac differentiation of injected stem cells and their integration in the cardiac muscle to compensate for the lost cardiomyocytes and replace endothelial cells.

Despite this, indirect paracrine signaling imposed itself to be the most beneficial mechanism *in vivo* (49,50). Cardiovascular tissue is influenced by paracrine signaling and several pathways come into action: TGF-ß, vascular endothelial growth factor (VEGF), stromal cell-derived factor (SDF)-1 and epidermal growth factor (EGF) can be secreted by transplanted stem cells. This signaling will generate neovascularization, reduction of cardiomyocytes apoptosis, and recruitment of tissue-repairing cells (45–47)

MSCs transplantation therapy relies also on the immunomodulation effect triggered by the secretion of TFG- ß, and interleukin (IL)-10 allowing to reduce the inflammation and improving healing (48).

Finally, the mechanism by which mesenchymal stem cells trigger cardiac remodeling is still under investigation. Current research suggests that an increase in matrix metalloproteinase levels induces scar size reduction and demonstrates modulation of the phenotype of cardiac fibroblasts induced by MSCs in vitro (51,52).

Nevertheless, additional studies are required to go further on the existence of the potential anti-inflammatory activity as well as the extent of immunomodulation produced by MSCs.

5. JUSTIFICATION AND HYPOTHESIS

JUSTIFICATION

Despite, significant improvements in MI prognosis over the past years, major public health issues remain stemming from MI events, causing significant mortality and chronic disabilities (18). The inability of the human heart to regenerate itself led research to focus on ways to induce cardiac repair through adult progenitor stem cells. The first promising preclinical study results were obtained in early 2000's observing the enhancement of ventricular function and repair of cardiac tissue through BMMSCs transplantation (53,54).

The increasing search studies for BMMSCs-like cells directed investigation toward the discovery of a variety of adult mesenchymal stem cells in almost every organ and tissues in the body (2–4).

Gronthos *et al.* (6) isolated the first type of dental stem cell from the human dental pulp: DPSCs. In 2003, SHED where identified by Dr. Songtao Shi's unpredictable discovery using his 6-years-old daughter's deciduous tooth (24).

Although dental stem cells lack the potency of their counterpart embryonic stem cells, they have the advantage of being free of the ethical and political concerns related to the destruction of human embryos. Dental stem cells can be easily obtained with minimal trauma, harvested from an individual, stored, and used for regeneration through autologous or allogenic transplant (55). Autologous cell transplant offers many advantages being a safe and effective technique overcoming histocompatibility issues and avoiding the transmission of viral diseases (56,57).

In 2008, in Valencia, Gandia *et al.* (58) evaluated the therapeutic potential of DPSCs in cardiac repair after myocardial infarction in rodents.

Ever since, the scientific interest for dental stem cells and non-dental regeneration kept increasing:

In 2016, “The Open study Of dental pulp stem cell Therapy in Humans” proposed a study protocol with the objective to evaluate safety and feasibility of autologous human adult dental pulp stem cell therapy in patients with chronic disability after stroke (59).

In 2020, at the peak of COVID-19 pandemic Ye *et al.* (60) investigated the therapeutic effect of dental pulp in treating severe pneumonia caused by COVID-19 infection.

In 2022, Suda *et al.* (61) explored the efficacy and safety of allogenic human dental pulp in patients with acute ischemic stroke.

All the previously mentioned arguments led to considerable hopes on the hypothesis that dental stem cells can be manipulated to achieve the ultimate goal of restoring damage myocardium.

Paradoxically, although teeth are considered to be non-essential for life, the promising stem cells held in their dental pulp may represent a new major key in potentially life-saving therapies and cardiac regenerative medicine. Thus, placing the humble tooth and dentists at the forefront in future developments of regenerative medicine.

To our knowledge, there is no systematic reviews published comparing the therapeutic potential of human dental pulp stem cells (DPSCs & SHED) to the one of hBMMSC for cardiac regeneration.

Consequently, it was considered justified to carry out a systematic review of the literature that evaluates both types of mesenchymal stem cells: human dental pulp stem cells (DPSCs or SHED) and hBMMSC, to analyze improvement of cardiac function, reduction of the infarct size, and angiogenesis induction in rodent models of myocardial infarction.

HYPOTHESIS

The working hypothesis of our study considers that DPSCs and SHED will achieve greater improvement in cardiac function, infarct size reduction, as well as induction of angiogenesis compared to hBMMSC in rodent models of myocardial infarction. Thus, making DPSCs and SHED a serious alternative source of stem cells for cardiac regeneration.

6. OBJECTIVES

General objective:

1. Evaluate improvement of cardiac function (through FS evolution) with DPSCs or SHED transplantation compared to hBMMSC transplantation in rodent models of myocardial infarction.

Specific objectives:

1. Measure the infarct size reduction induced by DPSCs or SHED compared to the one produced by hBMMSC in rodent models of myocardial infarction.

2. Analyse the ability of DPSCs or SHED to trigger angiogenesis through vascular density increase compared to hBMMSC in rodent models of myocardial infarction.

7. MATERIAL AND METHODS

The present systematic review was conducted following the PRISMA statement (2020) (Preferred Reporting Items for Systematic reviews and Meta-Analyses) (62).

7.1 PICO question identification

The Medline-PubMed database (United States National Library of Medicine), Web of Science, and Scopus were used to search for indexed articles on rodents with myocardial infarction who received dental stem cells transplantation (DPSCs or SHED) versus hBMMSC transplantation published up to January 2023 to answer the following question: In rodent models of myocardial infarction, does dental pulp stem cells (DPSCs or SHED) transplantation achieve greater improvement in cardiac function, reduction of the infarct size as well as induction of angiogenesis compared to hBMMSC?

This study question was set according to the structured PICO question. The question format was set as follows:

- **P** (Population): Rodent models of myocardial infarction
- **I** (Intervention): DPSCs or SHED transplantation
- **C** (Comparison): hBMMSC transplantation
- **O** (Outcomes):
    - O1: Improvement of cardiac function
    - O2: Infarct size reduction
    - O3: Induction of angiogenesis

7.2 Eligibility criteria

There was no restriction applied about the publication date.

- The inclusion criteria were:

  - **Type of studies**:

    Experimental animal studies ethically approved; studies published in English, French, or Spanish, up to January 2023.

  - **Type of population**:

    In vivo rodent models of myocardial infarction.

    Species: Rats, Mice, Rabbits.

    Sex: either male or female rodent.

  - **Type of intervention**:

    Transplantation of DPSCs or SHED or hBMMSC.

  - **Type of outcomes**:

    Studies measuring improvement of cardiac function as the primary outcome, reduction of the infarct size, and angiogenesis induction as secondary outcomes.

- The exclusion criteria were:

Peer reviews, reviews, letters to the editor, comments, expert opinion, editorials, randomized clinical trials, case series, studies on humans. In addition to this were excluded all studies conducted with non-human stem cells.

7.3 Information sources and search strategy

A search was carried out in the three following databases: PubMed, Scopus and Web of Science using the following keywords: “myocardial infarction”, “rodentia”, “acute myocardial infarction”, “chronic myocardial infarction”, “rodents”, “myocardial ischemia”, “reperfusion”, “human dental pulp stem cells”, “DPSC”, “dental stem cell therapy”, “stem cells from human exfoliated deciduous teeth”, “human Bone marrow-derived mesenchymal stem cells”, “BM-MSC”, “BMSC”, “human bone marrow multipotent mesenchymal stem cells”, “hMSCs", “cardiomyocytic differentiation” “cardiac repair”, “ventricular function”, “myocardial performance”, “cardiac function”. The keywords were combined with the Boolean operators AND and OR, as well as controlled termed (“MESH” for PubMed) to get the best and broadest search results.

The PubMed search executed on January 27th of 2023 was the following:

(((((((("Myocardial infarction" [MeSH Terms]) AND ("Rodentia"[MeSH Terms])) OR (Acute myocardial infarction)) OR (Chronic myocardial infarction)) AND (rodents) OR (myocardial ischemia))) AND ((((("Human dental pulp stem cells") OR ("DPSC")) OR (dental stem cell therapy)) OR ("Stem cells from human exfoliated deciduous teeth"))) OR (((((("Human Bone marrow-derived mesenchymal stem cells") OR ("BM-MSC")) OR ("BMSC")) OR (human bone marrow multipotent mesenchymal stem cells)) OR ("hMSCs"))) AND (((((("Cardiomyocytic differentiation") OR (cardiac repair)) OR ("ventricular function")) OR ("myocardial performance") OR ("cardiac function")) OR ("reperfusion"))

Results obtained: 366 articles

The Scopus search executed on January 27th of 2023 was the following:

(ALL("Myocardial infarction" AND "Rodentia" OR acute AND myocardial AND infarction OR chronic AND myocardial AND infarction AND rodents OR myocardial AND ischemia)) AND (ALL (human AND dental AND pulp AND stem AND cells OR dpsc OR dental AND stem AND cell AND therapy OR stem AND cells AND

from AND human AND exfoliated AND deciduous AND teeth)) OR (ALL (human AND bone AND marrow-derived AND mesenchymal AND stem AND cells OR bm-msc OR bmsc OR human AND bone AND marrow AND multipotent AND mesenc hymal AND stem AND cells OR hmscs)) AND (ALL (cardiomyocytic AND differentiation OR cardiac AND repair OR ventricular AND function OR myocardial AND performance OR cardiac AND function OR reperfusion))

Results obtained: 48 articles

The Web of Science executed on January 27th of 2023 was the following:

(((TS=(Myocardial infarction AND Rodentia OR Acute myocardial infarction OR Chronic myocardial infarction AND rodents OR myocardial ischemia OR "reperfusion")) AND TS=("Human dental pulp stem cells" OR "DPSC" OR dental stem cell therapy OR "Stem cells from human exfoliated deciduous teeth")) OR TS=("Human Bone marrow-derived mesenchymal stem cells" OR "BM-MSC" OR "BMSC" OR human bone marrow multipotent mesenchymal stem cells OR "hMSCs")) AND TS=("Cardiomyocytic differentiation" OR cardiac repair OR "ventricular function" OR "myocardial performance" OR "cardiac function" OR " reperfusion ")

Results obtained: 429 articles

A cross-search of possibly insightful publications was carried out for analysis. The authors of the papers were approached to acquire those that were not in full-text databases. The review was cleared of duplicate studies.

The search was finished with a check of the references listed in the bibliography of each study in order to find any suitable research that the initial search may have overlooked. The Journal of the American College of Cardiology and Medwell Journals publications were searched manually for scientific literature.

7.4 Study selection process

A three-phase selection process was carried out by two reviewers (EC, CC). Titles were analyzed in the preliminary stage, and irrelevant publications were discarded. The second phase was centered on abstracts, screening was performed, and the selection based itself on the type of study, type of stem cell transplants, type of model of myocardial infarction. The last phase examined the full-text and data were extracted using a pre-prepared data collection form to confirm the eligibility of the studies. Disagreements between reviewers, at each of the stages, were resolved by discussion, and, where necessary, a third reviewer was consulted.

The degree of agreement regarding the inclusion of potential studies was calculated by Cohen's kappa test (k-statistics) for the second and third stages of selection.

7.5 Data extraction

We extracted information from studies and organized it into tables according to authors with the year of publication, type of study, type of stem cell transplantation (DPSCs, SHED or hBMMSC), type of model of myocardial injury (rats, mice, rabbits), size of the sample, type of administration route (intravenous or intramyocardial), the time elapsed after transplantation (weeks), outcomes (improvement of cardiac function, infarct size reduction, vascular density evolution).

Primary variable:

- **Improvement of cardiac function:**

Echocardiographic parameters such as: left ventricular internal dimension at end-diastole (LVDd) and end-systole (LVDs) in millimeters, as well as fractional shortening (FS) in percentages, were analyzed 4 weeks after transplantation and compared to control groups. When non provided: fractional shortening, was calculated according to the following formula: FS= [[(LVDd - LVDs)/LVDd]*100] (63).

Secondary variables:

- **Infarct size reduction**

Cross-sections of excised hearts were stained, and the extension of the infarct size (infarct area/total left ventricular area) was expressed as percentage of the total left ventricular area. This calculation was done by making a mean of all slices from each heart. Results were analyzed in percentages and compared to the control group.

- **Induction of angiogenesis**

Vascular density was evaluated by immunostaining. The number of vessels per unit area (mm^2) was then expressed as percentages of change and compared to control groups.

7.6 Quality assessment

The quality assessment of experimental animal studies was based of the ARRIVE 2.0 (Animal Research: Reporting In Vivo Experiment) guidelines (64).

The risk of bias assessment was assessed by two reviewers (EC, CC) to analyze the methodological quality of the included articles.

The articles were examined, and bias was evaluated according to the evaluation of the 21 items (10 from the “Essential 10” and 11 from the “Recommended set”): if a publication satisfied all the subitems, it was scored as “reported” and given 2 points. If a publication did not satisfy any of the subitems, it was scored as “not reported” and given 0 points. If the details provided for the subitems were unclear, it was scored as “unclear” and given 1 point. In this way the study's quality was determined by applying a pre-established coefficient, which ranged from 0.8 to 1 for excellent quality, 0.5 to 0.8 for average quality, and less than 0.5 for poor quality. The coefficient was calculated by adding up the total points earned by each study and dividing it by the maximum possible points per study, which was 42.

Quality assessment by domain was also evaluated.

The degree of inter-examiner agreement in the assessment of methodological quality was determined using Cohen's kappa test, following the scale proposed by Landis and Koch (65).

7.7 Data synthesis

A descriptive analysis of the outcome variables was performed. Since the means calculated in the studies analyzed came from different samples size of rodents it was necessary to calculate the weighted mean with the objective of obtaining more accurate results. The means of values of primary variables were grouped by the research group (DPSCs, SHED, and hBMMSC). Due to the lack of randomized studies comparing all treatment groups, a meta-analysis was not possible, hence the results were concentrated on a descriptive analysis of the factors.

8. RESULTS

8.1 Study selection. Flow chart

A total of 843 studies were identified through the initial search process: Medline-PubMed (n=366), SCOPUS (n=48) and Web of Science (n=429). An additional study was obtained through a manual search (reference list and primary sources). Of these publications 24 were identified as potentially eligible by screening the titles. Screening the abstracts of these 24 publications led to the selection of 15 articles. Finally, full-text articles were exanimated, leading to the final selection of the 6 articles included in the present systematic review (Figure. 1). Information regarding the excluded articles (and the reasons for their exclusion) is presented in Table 1.

The k-value for inter-examiner agreement on the inclusion of studies was 0.87 (titles and abstracts) and 1.0 (full-texts) indicating "good" and "complete" agreement, respectively, according to Landis and Koch's criteria (65).

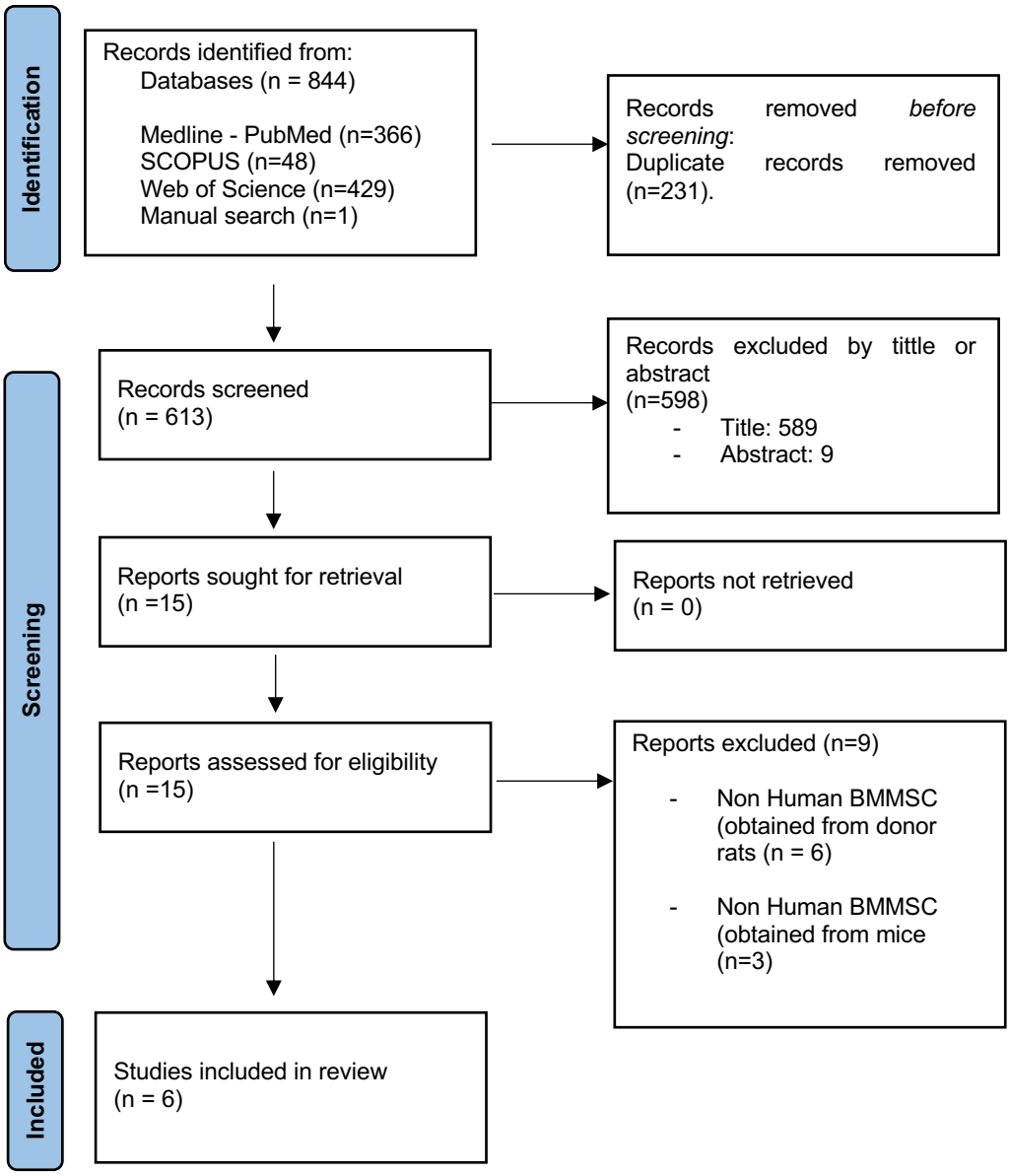

Figure.1. Search Flow diagram and title selection process of our systematic review.

Table 1. Excluded articles (and reason for exclusion) of our systematic review.

Authors. Year	Publication	Reason for exclusion
Olivares EL. 2004 (66)	Am J Physiol Heart Circ Physiol	Non-Human BMMSC (obtained from donor rats)
Loffredo FS. 2011 (67)	Cell Stem Cell	Non-Human BMMSC (obtained from donor rats)

Zhen H. 2012 (68)	Front Biosci (Landmark Ed)	Non-Human BMMSC (obtained from mice
Zhong Z. 2015 (69)	Int J Mol Med	Non-Human BMMSC (obtained from donor rats)
Li X. 2019 (70)	J Cell Physiol	Non-Human BMMSC (obtained from donor rats)
Carr CA. 2008 (71)	Am J Physiol Heart Circ Physiol	Non-Human BMMSC (obtained from donor rats)
Blume GG. 2021 (72)	Life (Basel)	Non-Human BMMSC (obtained from donor rats)
Xie DM. 2019 (73)	Stem Cell Res Ther	Non-Human BMMSC (obtained from mice
Omar AM. 2019 (74)	Oman Med J	Non-Human BMMSC (obtained from donor rats)

8.2 Analysis of included studies

Of the 6 articles selected in the present review, 1 describes the transplantation of SHED (75), 4 describe the transplantation of hBMMSC (76–79) and 1 describes the transplantation of DPSCs (80). All of them are animal experimental studies.

A total of 110 rodents were induced with myocardial infarction, of which 54 rodents were treated with human mesenchymal stem cells, including 8 rodents treated with SHED, 7 rodents treated with DPSCs and 39 rodents treated with hBMMSC.

Two studies used a rabbit model of myocardial infarction (75,76), 3 used a rat model (77,79,80) and 1 used a mouse model (78).

Regarding the administration route of mesenchymal stem cells, 5 studies used intramyocardial injection (76–80), and 1 study used and an intravenous injection (75).

All data were collected 4 weeks after transplantation as presented in Table 2.

Table 2. Characteristics of the reviewed studies

Authors. Year	Type of study	Type of transplanted cell	Model of MI	Sample size	Administration route	Time elapsed after transplantation
Petchdee *et al.*(75) 2014	Experimental animal study	Human SHED	Rabbit Model	n= 16 (Control group n=8, Cell treated group n=8)	Intravenous injection through rabbit marginal ear vein	4 weeks
Wang *et al.*(76) 2005	Experimental animal study	Human BMMSC	Rabbit Model	n= 25 (Control group n=13, Cell treated group n=12)	Intramyocardial injection into the border area of the ischemic myocardium	4 weeks
Gandia *et al.*(80) 2008	Experimental animal study	Human DPSCs	Rat Model	n= 16 (Control group n=9, Cell treated group n=7)	Intramyocardial transplantation in five injections at five points of the infarct border	4 weeks
Liu *et al.*(77) 2008	Experimental animal study	Human BMMSC	Rat Model	n=23 (Control group n=12, Cell treated group n=11)	Intramyocardial injection at the left anterior free wall	4 weeks
Shyu *et al.*(78) 2006	Experimental animal study	Human BMMSC	Mouse model	n=14 (Control group n=7, Cell treated group n=7)	Intramyocardial injection	4 weeks
Rasmussen *et al.*(79) 2014	Experimental animal study	80 years old Human BMMSC	Rat Model	n=16 (Control group n=7 Cell treated group n=9)	Intramyocardial injection	4 weeks

8.3 Quality assessment and risk of bias

In this review, two studies were rated as having excellent quality (78,80), while the remaining four studies were classified as having average quality (75–77,79)as presented in Figure 2. The item that posed the highest risk of bias was housing and husbandry, as indicated in Figure 3. The kappa value (Cohen kappa test) for inter-reviewer agreement on methodological quality was 0.9 according to the Landis & Koch scale (65).

	Soontaree *et al.* 2014	Wang *et al.* 2005	Gandia *et al.* 2008	Liu *et al.* 2008	Shyu *et al.* 2006	Rasmussen *et al.* 2014
1. Study design For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	2	2	2	2	2	2
2. Sample size a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	1	1	2	1	2	1
3. Inclusion and exclusion criteria a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	2	2	2	2	2	2
4. Randomization a. State whether randomization was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomization sequence. b. Describe the strategy used to minimize potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	1	1	1	1	1	1
5. Blinding Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	0	0	2	2	2	2
6. Outcome measures a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	2	2	2	2	2	2
7. Statistical methods a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	2	2	2	2	2	2
8. Experimental animals a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	1	1	1	1	1	1
9. Experimental procedures For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatization periods). d. Why (provide rationale for procedures).	1	1	1	1	1	1

10. Results For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	2	2	2	2	2	2
11. Abstract Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	2	2	1	1	1	2
12. Background a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	2	2	2	2	2	2
13. Objectives Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	2	2	2	2	2	2
14. Ethical statement Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	2	2	2	2	2	2
15. Housing and husbandry Provide details of housing and husbandry conditions, including any environmental enrichment.	0	0	0	0	0	0
16. Animal care and monitoring a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	1	1	1	1	1	1
17. Interpretation/ scientific implications a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	2	2	2	2	2	2
18. Generalizability/ translation Comment on whether, and how, the findings of this study are likely to generalize to other species or experimental conditions, including any relevance to human biology (where appropriate).	2	2	2	2	2	2
19.Protocol registration Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	1	1	1	1	1	1
20. Data access Provide a statement describing if and where study data are available.	2	2	2	2	2	2
21. Declaration of interests a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	2	0	2	2	2	2
Coefficient:	32/42 **0,76**	30/42 **0,71**	34/42 **0,81**	33/42 **0,78**	34/42 **0,81**	34/42 **0,76**

Quality:	Average	Average	Excellent	Average	Excellent	Average

Figure.2. Quality coefficients of the studies reviewed according to ARRIVE 2.0 guidelines.

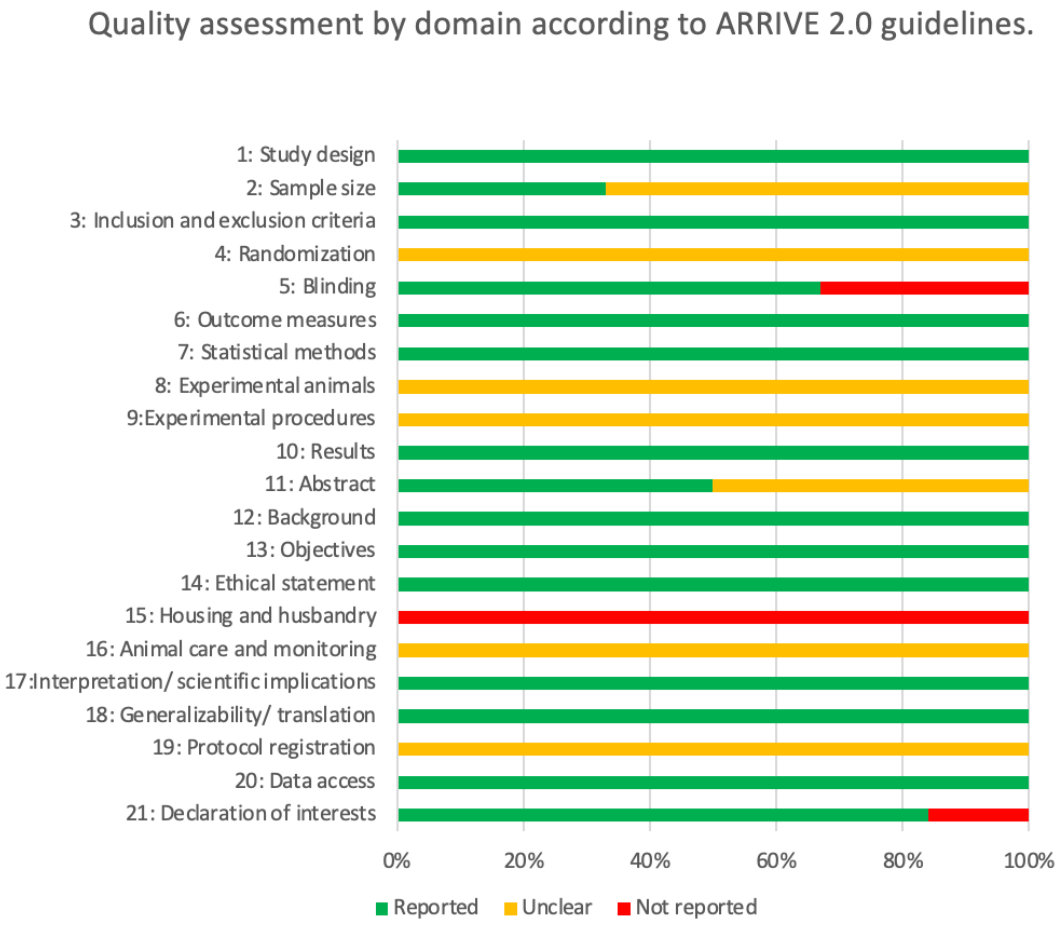

Figure.3. Quality assessment by domain according to ARRIVE 2.0 guidelines.

8.4 Outcomes

This systematic review focused on 3 main outcomes: improvement of cardiac function, infarct size reduction, and induction of angiogenesis. A general synthesis of the outcomes obtained from the studies is presented in Table 3. Each outcome will be individually presented in the next sections.

Table 3. Outcomes synthesis by study

Authors. Year	Type of study	Type of transplanted cell	Model of MI	Sample size	Administration route	Time elapsed after MI induction	Outcomes
Petchdee *et al.*(75) 2014	Experimental animal study	Human SHED	Rabbit Model	n=16	Intravenous injection through rabbit marginal ear vein	4 weeks	1) ✗ Non-significant results in cardiac function 2) ✓ Significant infarct size reduction 3) ✓ Observed angiogenesis
Wang *et al.*(76) 2005	Experimental animal study	Human BMMSC	Rabbit Model	n=25	Intramyocardial injection injecting into the border area of the ischemia myocardium.	4 weeks	1) ✓ Significant improvement in cardiac function 2) ✓ Significant infarct size reduction
Gandia *et al.*(80) 2008	Experimental animal study	Human DPSCs	Rat Model	n=16	Intramyocardial transplantation in five injections at five points of the infarct border	4 weeks	1) ✓ Significant improvement in cardiac function 2) ✓ Significant infarct size reduction 3) ✓ Significant vascular density increase
Liu et al.(77) 2008	Experimental animal study	Human BMMSC	Rat Model	n=23	Intramyocardial injection at the left anterior free wall	4 weeks	1) ✓ Significant improvement in cardiac function 2) ✓ Significant infarct size reduction 3) ✓ Significant vascular density increase
Shyu et al.(78) 2006	Experimental animal study	Human BMMSC	Mouse model	n=14	Intramyocardial injection	4 weeks	1) ✓ Significant improvement in cardiac function 2) ✓ Significant infarct size reduction 3) ✓ Significant vascular density increase
Rasmussen *et al.*(79) 2014	Experimental animal study	80 years old Human BMMSC	Rat Model	n=16	Intramyocardial injection	4 weeks	1) ✗ Non-significant results in cardiac function 2) ✗ Non-significant results in infarct size reduction 3) ✗ Non-significant results in vascular density increase

8.4.1 Improvement of cardiac function

Regarding the improvement of cardiac function all 6 studies included in the review (75–80) provided data about FS. The studies conducted by Petchdee *et al.* (75) with SHED and Rasmussen *et al.* (79) with hBMMSC did not provide significant differences in FS evolution between the control group and treated groups.

Gandia *et al*. (80) observed a significant increase in FS of 36.95% compared to the control group 4 weeks after intramyocardial injection of DPSCs at the infarct border. The control group showed a significant deterioration in cardiac function and internal ventricular dimensions.

The weighted mean increase in FS in the hBMMSC group was 27.13% compared to the control group 4 weeks after intramyocardial injection (76–79). The highest increase in FS was observed by Liu *et al.* (77) with 69.84%. The lowest increase was observed by Wang *et al.* (76) with an increase of 6.68%. The control groups showed a significant decrease in cardiac function and ventricular dimensions. The only study that did not show a significant improvement in cardiac function with hBMMSC was the one conducted by Rasmussen *et al.* (79).

SHED on the other hand did not show a significant improvement in cardiac function (75).

The greatest improvement in cardiac function was achieved by DPSCs group (80) with a significant difference (p=0,010) compared to hBMMSC.

Descriptive results of echocardiographic parameters are shown in Table 4.

Table 4. Echocardiographic parameters of LVDd, LVDs and FS by study

DPSCs	**LVDd (mm) Control 4 weeks**	**LVDd (mm) DPSCs 4 weeks**	**LVDs (mm) Control 4 weeks**	**LVDs (mm) DPSCs 4 weeks**	**FS% Control 4 weeks**	**FS% DPSCs 4 weeks**	**FS evolution %**
Gandia *et al.* (80)	7.60 ± 0.18	7.26 ± 0.32	5.63 ± 0.23	4.94 ± 0.24	24.2 ± 1.6	32.9 ± 1.6	+ 36.95 %
Weighted mean DPSCs: + 35.95% FS							
hBMMSC	**LVDd (mm) Control 4 weeks**	**LVDd (mm) hBMMSC 4 weeks**	**LVDs (mm) Control 4 weeks**	**LVDs (mm) hBMMSC 4 weeks**	**FS% Control 4 weeks**	**FS% hBMMSC 4 weeks**	**FS evolution %**
Wang *et al.* (76)	15.4 ± 1.4	11.2 ± 1.8	9.7 ± 0.5	6.7 ± 0.5	37.7 ± 3.28	40.2 ± 3,3	+ 6.68%
Liu *et al.* (77)	7.8 ± 0.2	6.5 ± 0.1	5.4 ± 0.6	3.0 ± 0.1	31.5 ± 6.5	53.5 ± 1.7	+ 69.84%
Shyu *et al.* (78)	4.6 ± 0.2	3.6 ± 0.1	3.1 ± 0.3	2.2 ± 0.1	34.2 ± 2.3	44.4 ± 2.3	+ 30.01%
Rasmussen *et al.* (79)	-	-	-	-	-	-	Non-significant= 0%
Weighted mean hBMMSC: +27.13% FS							
SHED	**LVDd (mm) Control 4 weeks**	**LVDd (mm) SHED 4 weeks**	**LVDs (mm) Control 4 weeks**	**LVDs (mm) SHED 4 weeks**	**FS% Control 4 weeks**	**FS% SHED 4 weeks**	**FS evolution %**
Petchdee *et al*. (75)	-	-	-	-	-	-	Non-significant= 0%

8.4.2 Infarct size reduction

The size of the infarct area was evaluated by 5 studies (75,77–80). All three types of stem cells treatment: DPSCs, SHED and hBMMSC showed significant results (p<0,5) in the reduction of the infarct size.

In the study conducted by Gandia *et al.* (80) DPSCs showed a mean infarct size reduction of 25% compared to the control group, 4 weeks after intramyocardial injection at the border of the infarct. Petchdee *et al.* (75) observed a 45.2% reduction of the infarct size with the intravenous injection of SHED.

Regarding the hBMMSC group, Rasmussen *et al.*(79) conducted the only study within this group that did not achieve a significant reduction in infarct size. The weighted mean reduction with hBMMSC was 18.67%. The greatest reduction in infarct size was achieved by SHED with a 45.2%, followed by the DPSCs group with a reduction of 25% and the least reduction was achieved by hBMMSC group with 18.67%. Descriptive results on infarct size reduction are shown in Table 5.

Table 5. Infarct size reduction results

	Infarct size Control group after 4 weeks (mm)	**Infarct size Treated group after 4 weeks (mm)**	**Infarct size evolution %**
DPSCs			
Gandia *et al.* (80)	21.2 ± 1.6 %	15.9 ± 1.7 %	-25%
Weighted mean reduction of infarct size: - 25%			
SHED			
Petchdee *et al.* (75)	19.9 ± 0.03 %	10.9 ± 0.02 %	-45.2%
Weighted mean reduction of infarct size: - 45.2%			
hBMMSC			
Liu *et al.* (77)	54.9±3.3%	35.4±3.4%	-35.5%
Rasmussen *et al.* (79)	-	-	Non-significant = 0%
Shyu *et al.* (78)	66.1±1.5%	58.1±1.1%	-12.1%
Weighted mean reduction of infarct size: -18.67%			

8.4.3 Induction of angiogenesis

Five studies analyzed the induction of angiogenesis by measuring the increase in vascular density at the infarct zone (75,77–80).

DPSCs induced angiogenesis, resulting in the formation of functional rat-derived blood vessels. There was a 67.51% increase in vascular density compared to the control group (80).

Petchdee *et al.* (75) observed an increase in vascular density through histological sections stained with Hematoxylin & Eosin (H&E). A quantitative comparison was not possible as exact values were not provided.

The weighted mean increase for the hBMMSC group was 34.57%, with the highest increase of 84.64% obtained by Liu *et al.* (77). Once again, the study conducted by Rasmussen *et al.* (79) did not obtain significant results in the induction of angiogenesis. Descriptive results of vascular density evolution are presented in Table 6.

Table 6. Descriptive results of vascular density evolution

	Control group vascular density after 4 weeks number of vessels per unit area (mm^2)	**Treated group vascular density after 4 weeks number of vessels per unit area (mm^2)**	**Vascular density evolution %**
DPSCs			
Gandia *et al.* (80)	518 ± 115/mm^2	868 ± 85/mm^2	+ 67.51%
Weighted mean increase of vascular density: + 67.51%			
SHED			
Petchdee *et al.* (75)	-	-	Increased number of capillaries based on H&E staining but not quantified.
Weighted mean increase of vascular density: Not quantified			
hBMMSC			
Liu *et al.* (77)	144 ± 16/mm^2	938±42/mm^2	+84.64%
Rasmussen *et al.* (79)	-	-	Non-significant = 0%
Shyu *et al.* (78)	439±27/mm^2	1119±17/mm^2	+60.8%
Weighted mean increase of vascular density: + 34.57%			

DISCUSSION

The present systematic review aims to provide evidence-based information on the outcomes produced by DPSCs, SHED or hBMMSC transplantation in myocardial regeneration. The general objective was to evaluate improvement of cardiac function (through FS evolution) with DPSCs or SHED transplantation compared to hBMMSC transplantation in rodent models of myocardial infarction. Additionally, specific objectives of this systematic review included: measuring the reduction of the infarct size and analyzing the induction of angiogenesis resulting from DPSCs or SHED transplantation in comparison to hBMMSC transplantation in rodent models of myocardial infarction.

8.5 Improvement of cardiac function

Due to the limited capacity of cardiac muscle to proliferate, regenerative treatments are in high demand as a novel treatment approach. Although pharmacological and non-pharmacological treatments were created, their ability to effectively treat patients with severe heart failure after myocardial infarction is still limited. Additionally, the lack of organ donors limits the number of heart transplants that can be performed. Thus, autologous stem cell induced heart regeneration with a great deal of potential to offer (81–83).

The results of this systematic review based on six scientific investigations revealed a greater improvement in cardiac function with DPSCs intramyocardial injection compared to hBMMSC and SHED. Gandia *et al.* (80) not only observed improvement of cardiac function but also observed ventricular remodeling and enhancement of the regional contractility. The cardiac repair occurred without cell differentiation.

The lack of results in cardiac regeneration observed by Rasmussen *et al.* (79) following hBMMSC transplantation may be attributed to the fact that the stem cells used in the experiment were derived from an 80-year-old donor. Indeed, Fan *et al.*(84) and Liu *et*

al.(85) discussed the importance of advanced age on the cardioprotective and regenerative capacities of mesenchymal stem cells. Both studies showed that age had a substantial impact on the ability of hBMMSC to regenerate tissues. Young hBMMSC transplants enhanced functional outcomes following a MI by stopping matrix breakdown and encouraging angiogenesis (84,85).

In 2017, Song *et al.* (86) conducted a study where they further explored the rejuvenation of hBMMSC and observed its effectiveness. They found out that cardiac performance improved after myocardial infarction was obtained in mice hearts by transplanting neuron-derived neurotrophic factor over-expressing aged hBMMSC, which enhanced the survival of the implanted stem cell.

Regarding SHED although they did not provide significant results in improving cardiac function, they validated the safety of SHED transplantation after myocardial infarction in a rabbit model (75). A study conducted by Yamaguchi *et al.* (87) provided the first evidence that SHED conditioned-medium conferred resistance to acute ischemic damage in the heart: as a matter of fact, SHED conditioned-medium significantly increased ventricular FS, suppressed apoptosis and inflammation in the ischemic heart of mice 7 days after myocardial infarct induction.

About conditioned medium and bone marrow-derived mesenchymal stem cells, a study conducted by Li *et al*.(88) investigated the use of medium derived from bone marrow mesenchymal stem cells and concluded that it could also serve as a promising treatment to protect cardiomyocytes from hypoxia and reoxygenation injuries.

The previous data presented strongly suggest that the observed benefits after DPSCs, SHED, and hBMMSC transplantation could be attributed to the secretion of paracrine factors (89). Furthermore, it was demonstrated that bone marrow-derived c-kit cells enhance cardiac function by increasing VEGF levels and modulating the cardiac ratio of angiopoietin-1 to angiopoietin-2 using the c-kit mutant KitW/KitW-v mice (89–92).

8.6 Reduction of the infarct size

Both dental stem cells (DPSCs and SHEDs) exhibited a higher percentage of infarct size reduction compared to hBMMSC.

This reduction can be explained through ultrastructural examination of left ventricular wall and peri-infarct zones, which showed an increase in cardiomyocyte bundles that decreased the infarct size and encouraged a higher fraction of myofibroblast. Myofibroblasts are linked to the recovery of ischemic wounds, and their ontogenesis is connected to the morphological alterations seen in fibroblasts under stress-strain (75,80).

While DPSCs did not differentiate into cardiomyocytes, engrafted hBMMSC presented cardiomyocytic differentiation conferring them the ability to survive long-term in cardiac tissue (76–78,80). An *in vitro* study conducted by Kittivarakam *et al*. (93) showed that SHED could differentiate into functional cardiomyocytes and were able to proliferate on electrospun scaffolds. We can also add that through longer periods of time allogenic bone marrow mesenchymal stem cells required between 3 to 6 months to express muscle markers, thus not ruling out the possibility that longer periods of transplantation may be necessary to induce the expression of cardiac markers in transplanted DPSCs (94).

Di Scipio *et al*. (95) investigated the impact of injured cardiomyocytes on DPSCs and demonstrated *in vitro* that damaged cardiomyocytes could stimulate DPSCs migration and adhesion to the extracellular matrix.

Donor's age impaired the ability of hBMMSC to reduce the infarct size: Rasmussen *et al.* (79) did not observe any result using the hBMMSC from the 80-year-old donor.

8.7 Induction of angiogenesis

The heart is an organ whose function critically depends on its supply of oxygen. The angiogenic properties of dental stem cells was already proven and used for dental regeneration (96–98).

In this review we observed that DPSCs achieved a greater angiogenesis induction with a higher percentage of vascular density increase compared to hBMMSC. Angiogenesis triggered by SHED was not compared because the study did not provide quantitative data.

Fazel *et al.* (99) correlated angiogenesis produced by hBMMSC with the repopulation of infarct area with myofibroblasts in a mouse model of cardiomyopathy. Shyu *et al.* (78) demonstrated that hBMMSC presented a superior potential in enhancing angiogenesis than VEGF and angiogenic growth factors after myocardial infarction in mice.

The age of the donor of hBMMSC also played a role in the induction of angiogenesis: Rasmussen *et al.* (79) observed that 80-year-old hBMMSC did not provide any significative result in angiogenesis induction. Fan *et al.* (84), on the other hand, observed that VEGF and protein levels were significantly elevated in the myocardium after young hBMMSC were transplanted.

Regarding the mechanisms of how dental stem cells can trigger angiogenesis the same previously mentioned paracrine mechanisms are highly susceptible to be involved. DPSCs can secrete angiogenic growth factors including VEGF, fibroblast growth factor (bFGF) and platelet derived growth factor (PDGF) which are sufficient to mediate the formation of a network of tubular structure of endothelial cells thus indicating angiogenic stimulation (89,96–98,100,101).

8.8 Limitations

The present review revealed a lack of experimental animal studies focused on dental pulp stem cells as only one study is at the present moment focused on DPSCs and myocardial infarction (80). At the moment only one similar study focused on SHED is available (75). For this reason, the results of the present systematic review must be interpreted with caution.

Another limitation is the lack of uniformity in the data collected about cardiac function measurement: studies about dental stem cells lacked data about ejection fraction figures, and fractional shortening was not always directly measured. Despite this, data about LVDd and LVDs allowed us to calculate FS and thus compare improvement achieved by each group of cells (75,80). A more complete analysis of cardiac function would have required ejection fraction measurements.

Similarly, there is a lack of uniformity in the way of measuring capillary density. In some studies it was obtained through immunostaining, whereas in others was assessed from photomicrographs by computerized image analysis.

The age of donor cells also influenced the results and drastically lowered the weighted mean results of the hBMMSC group. The lack of information about the age of donors could not allow us to differentiate results inside the hBMMSC group (79,84–86).

Another limitation was the lack of follow-up time to be able to detect potential oncogenic side effects or DPSCs cardiomyocytic differentiation.

8.9 Clinical implications and future perspectives

Dental pulp stem cells represent a substantial alternative for non-dental regeneration and treatment of various inflammatory diseases thanks to their immunomodulatory properties and differentiation potential.

Pre-clinical implications in experimental animal studies provided successful promising results opening the way to further research encouraging clinical trials to validate the safety and efficacy of dental pulp stem cell transplantation.

In the future, dental stem cells may also be combined with conditioned medium, other type of stem cells, scaffolds and growth factors that may improve their properties in myocardial regeneration.

Current improvements in dental pulp stem cell banking along with their abundance, easy accessibility, low tumorigenicity and ethical acceptability place the humble tooth as a strong contender for inter-regenerative medicine.

9. CONCLUSION

General conclusion:

1. Both DPSCs and hBMMSC transplantation demonstrated improvement in cardiac function, with a higher fractional shortening improvement observed in the DPSCs group. However, SHED transplantation did not significantly improve cardiac function in rodent models of myocardial infarction.

Specific conclusions:

1. SHED transplantation resulted in the most significant reduction of the infarct size compared to hBMMSC or DPSCs transplantation in rodent models of myocardial infarction.

2. Both SHED and DPSCs transplantation triggered angiogenesis. Vascular density increase being the highest in the DPSCs group compared to hBMMSC in rodent models of myocardial infarction.

10. BIBLIOGRAPHY

1. Aly LAA. Stem cells: Sources, and regenerative therapies in dental research and practice. World J Stem Cells. 2015 Aug 26;7(7):1047–53.
2. Wang M, Yuan Q, Xie L. Mesenchymal stem cell-based immunomodulation: Properties and clinical application. Vol. 2018, Stem Cells International. Hindawi Limited; 2018.
3. Baksh D, Song L, Tuan RS. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. J Cell Mol Med. 2004;8(3):301–16.
4. Porada C, Zanjani E, Almeida-Porada G. Adult mesenchymal stem cells: a pluripotent population with multiple applications. Curr Stem Cell Res Ther. 2006 Nov 8;1(3):365–9.
5. Yamada Y, Nakamura-Yamada S, Kusano K, Baba S. Clinical potential and current progress of dental pulp stem cells for various systemic diseases in regenerative medicine: A concise review. Vol. 20, International Journal of Molecular Sciences. MDPI AG; 2019.
6. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000 Dec 5;97(25):13625–30.
7. Volponi AA, Pang Y, Sharpe PT. Stem cell-based biological tooth repair and regeneration. Trends Cell Biol. 2010 Dec;20–206(12–6):715.
8. Patil R, Kumar BM, Lee WJ, Jeon RH, Jang SJ, Lee YM, et al. Multilineage potential and proteomic profiling of human dental stem cells derived from a single donor. Exp Cell Res. 2014 Jan 1;320(1):92–107.
9. Ishkitiev N, Yaegaki K, Calenic B, Nakahara T, Ishikawa H, Mitiev V, et al. Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. J Endod. 2010 Mar;36(3):469–74.
10. Armiñán A, Gandía C, Bartual M, García-Verdugo JM, Lledó E, Mirabet V, et al. Cardiac differentiation is driven by NKX2.5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. Stem Cells Dev. 2009 Jul 1;18(6):907–17.
11. Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells. 2008 Jul 1;26(7):1787–95.
12. Heng BC, Lim LW, Wu W, Zhang C. An Overview of Protocols for the Neural Induction of Dental and Oral Stem Cells In Vitro. Tissue Eng Part B Rev. 2016 Jun 1;22(3):220–50.
13. Saghiri MA, Asatourian A, Sorenson CM, Sheibani N. Role of angiogenesis in endodontics: contributions of stem cells and proangiogenic and antiangiogenic factors to dental pulp regeneration. J Endod. 2015 Jun 1;41(6):797–803.
14. Stevens A, Zuliani T, Olejnik C, LeRoy H, Obriot H, Kerr-Conte J, et al. Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev. 2008;17(6):1175–84.

15. Carnevale G, Riccio M, Pisciotta A, Beretti F, Maraldi T, Zavatti M, et al. In vitro differentiation into insulin-producing β-cells of stem cells isolated from human amniotic fluid and dental pulp. Dig Liver Dis. 2013 Aug;45(8):669–76.
16. Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endod. 2009 Nov;35(11):1536–42.
17. Gioventù S, Andriolo G, Bonino F, Frasca S, Lazzari L, Montelatici E, et al. A novel method for banking dental pulp stem cells. Transfus Apher Sci. 2012 Oct;47(2):199–206.
18. Kim SJ. Global awareness of myocardial infarction symptoms in general population. Vol. 51, Korean Circulation Journal. Korean Society of Cardiology; 2021.
19. Favreau-Lessard AJ, Sawyer DB. Cardiac Regeneration and Stem Cells as Therapy for Heart Disease. Encyclopedia of Cardiovascular Research and Medicine. 2018;468–74.
20. Koninckx R, Hensen K, Danils A, Moreels M, Lambrichts I, Jongen H, et al. Human bone marrow stem cells co-cultured with neonatal rat cardiomyocytes display limited cardiomyogenic plasticity. Cytotherapy. 2009;11(6):778–92.
21. Igarashi A, Segoshi K, Sakai Y, Pan H, Kanawa M, Higashi Y, et al. Selection of common markers for bone marrow stromal cells from various bones using real-time RT-PCR: Effects of passage number and donor age. Tissue Eng. 2007 Oct 1;13(10):2405–17.
22. Rai S, Kaur M, Kaur S, Arora S. Redefining the potential applications of dental stem cells: An asset for future. Vol. 18, Indian Journal of Human Genetics. 2012. p. 276–84.
23. Armiñán A, Gandía C, Bartual M, García-Verdugo JM, Lledó E, Mirabet V, et al. Cardiac differentiation is driven by nkx2.5 and gata4 nuclear translocation in tissue-specific mesenchymal stem cells. Stem Cells Dev. 2009 Jul 1;18(6):907–17.
24. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A. 2003 May 13;100(10):5807–12.
25. Sloan AJ, Waddington RJ. Dental pulp stem cells: What, where, how? Int J Paediatr Dent. 2009 Jan;19(1):61–70.
26. Huang GTJ, Gronthos S, Shi S. Critical reviews in oral biology & medicine: Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in Regenerative Medicine. Vol. 88, Journal of Dental Research. 2009. p. 792–806.
27. Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira L V., et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs. 2006 Apr;184(3–4):105–16.
28. Hilkens P, Driesen RB, Wolfs E, Gervois P, Vangansewinkel T, Ratajczak J, et al. Cryopreservation and banking of dental stem cells. Adv Exp Med Biol. 2016 Nov 1;951:199–235.
29. Arora V, Arora P, Munshi A. Banking stem cells from human exfoliated deciduous teeth (SHED): saving for the future. J Clin Pediatr Dent. 2009 Jul 1;33(4):289–94.

30. Woods EJ, Perry BC, Hockema JJ, Larson L, Zhou D, Goebel WS. Optimized cryopreservation method for human dental pulp-derived stem cells and their tissues of origin for banking and clinical use. Cryobiology. 2009 Oct;59(2):150–7.
31. Kumar A, Bhattacharyya S, Rattan V. Effect of uncontrolled freezing on biological characteristics of human dental pulp stem cells. Cell Tissue Bank. 2015 Dec 1;16(4):513–22.
32. Zhurova M, Woods EJ, Acker JP. Intracellular ice formation in confluent monolayers of human dental stem cells and membrane damage. Cryobiology. 2010 Aug;61(1):133–41.
33. Hunt CJ. Cryopreservation of Human Stem Cells for Clinical Application: A Review. Transfusion Medicine and Hemotherapy. 2011 Apr;38(2):107.
34. Ducret M, Fabre H, Degoul O, Atzeni G, McGuckin C, Forraz N, et al. Manufacturing of dental pulp cell-based products from human third molars: current strategies and future investigations. Front Physiol. 2015 Aug 6;6(Aug):213–213.
35. Papaccio G, Graziano A, D'Aquino R, Graziano MF, Pirozzi G, Menditti D, et al. Long-term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: a cell source for tissue repair. J Cell Physiol. 2006 Aug;208(2):319–25.
36. Kosuge M, Kimura K, Ishikawa T, Ebina T, Hibi K, Tsukahara K, et al. Differences between men and women in terms of clinical features of ST-segment elevation acute myocardial infarction. Circ J. 2006;70(3):222–6.
37. Erhardt L, Herlitz J, Bossaert L, Halinen M, Keltai M, Koster R, et al. Task force on the management of chest pain. Eur Heart J. 2002 Aug;23(15):1153–76.
38. Jenča D, Melenovský V, Stehlik J, Staněk V, Kettner J, Kautzner J, et al. Heart failure after myocardial infarction: incidence and predictors. ESC Heart Fail. 2021 Feb 1;8(1):222–37.
39. Cheng P, Zhu H, Witteles RM, Wu JC, Quertermous T, Wu SM, et al. Cardiovascular Risks in Patients with COVID-19: Potential Mechanisms and Areas of Uncertainty.
40. Pearlman JD, Triulzi MO, King ME, Newell J, Weyman AE. Limits of normal left ventricular dimensions in growth and development: analysis of dimensions and variance in the two-dimensional echocardiograms of 268 normal healthy subjects. J Am Coll Cardiol. 1988;12(6):1432–41.
41. Nanda NC, Miller AP. Principles of intracardiac echocardiography. Journal of Interventional Cardiac Electrophysiology. 2005 Aug;13(SUPPL. 1):7–10.
42. St. John Sutton MG, Sharpe N. Left Ventricular Remodeling After Myocardial Infarction. Circulation. 2000 Jun 27;101(25):2981–8.
43. Çağlı K, Gölbaşı Z. [3-dimensional echocardiography part I: Basic principles]. Turk Kardiyol Dern Ars. 2019;47(6):516–20.
44. Choi YH, Kurtz A, Stamm C. Mesenchymal stem cells for cardiac cell therapy. Hum Gene Ther. 2011 Jan 1;22(1):3–17.
45. Redgrave RE, Tual-Chalot S, Davison BJ, Singh E, Hall D, Amirrasouli MM, et al. Cardiosphere-Derived Cells Require Endoglin for Paracrine-Mediated Angiogenesis. Stem Cell Reports. 2017 May 5;8(5):1287.
46. Chimenti I, Smith RR, Li TS, Gerstenblith G, Messina E, Giacomello A, et al. Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice. Circ Res. 2010 Mar 19;106(5):971–80.

47. Hodgkinson CP, Bareja A, Gomez JA, Dzau VJ. Emerging Concepts in Paracrine Mechanisms in Regenerative Cardiovascular Medicine and Biology. Circ Res. 2016 Jan 8;118(1):95–107.
48. Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. Cell Mol Life Sci. 2017 Jul 1;74(13):2345–60.
49. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res. 2008 Nov 21;103(11):1204–19.
50. Menasché P, Vanneaux V. Stem cells for the treatment of heart failure. Curr Res Transl Med. 2016 Apr 1;64(2):97–106.
51. Rota M, Padin-Iruegas ME, Misao Y, De Angelis A, Maestroni S, Ferreira-Martins J, et al. Local activation or implantation of cardiac progenitor cells rescues scarred infarcted myocardium improving cardiac function. Circ Res. 2008 Jul 3;103(1):107–16.
52. Mias C, Lairez O, Trouche E, Roncalli J, Calise D, Seguelas MH, et al. Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction. Stem Cells. 2009 Nov;27(11):2734–43.
53. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet. 2003 Jan 4;361(9351):45–6.
54. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate infarcted myocardium. Nature. 2001 Apr 5;410(6829):701–5.
55. Marion NW, Mao JJ. Mesenchymal stem cells and tissue engineering. Methods Enzymol. 2006;420:339–61.
56. Nakashima M, Iohara K. Recent Progress in Translation from Bench to a Pilot Clinical Study on Total Pulp Regeneration. J Endod. 2017 Sep 1;43(9S):S82–6.
57. Xuan K, Li B, Guo H, Sun W, Kou X, He X, et al. Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. Sci Transl Med. 2018 Aug 22;10(455).
58. Gandia C, Armiñan A, García-Verdugo JM, Lledó E, Ruiz A, Miñana MD, et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells. 2008 Mar 1;26(3):638–45.
59. Nagpal A, Kremer KL, Hamilton-Bruce MA, Kaidonis X, Milton AG, Levi C, et al. TOOTH (The Open study Of dental pulp stem cell Therapy in Humans): Study protocol for evaluating safety and feasibility of autologous human adult dental pulp stem cell therapy in patients with chronic disability after stroke. Int J Stroke. 2016 Jul 1;11(5):575–85.
60. Ye Q, Wang H, Xia X, Zhou C, Liu Z, Xia ZE, et al. Safety and efficacy assessment of allogeneic human dental pulp stem cells to treat patients with severe COVID-19: structured summary of a study protocol for a randomized controlled trial (Phase I / II). Trials. 2020 Jun 12;21(1).
61. Suda S, Nito C, Ihara M, Iguchi Y, Urabe T, Matsumaru Y, et al. Randomised placebo-controlled multicentre trial to evaluate the efficacy and safety of JTR-161, allogeneic human dental pulp stem cells, in patients with Acute Ischaemic stRoke (J-REPAIR). BMJ Open. 2022 May 24;12(5).

62. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. International Journal of Surgery. 2010;8(5):336–41.
63. C T, H LL, O HD, R BK, K OJ, J RR, et al. New Index of Combined Systolic and Diastolic Myocardial Performance: A Simple and Reproducible Measure of Cardiac Function-A Study in Normals and Dilated Cardiomyopathy. J Cardiol. 1995;26(6):357–66.
64. Percie Du Sertid N, Hurst V, Ahluwaliaid A, Alamid S, Aveyid MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. 2020;
65. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics. 1977 Jun 1;33(2):363–74.
66. Olivares EL, Ribeiro VP, Werneck De Castro JPS, Ribeiro KC, Mattos EC, Goldenberg RCS, et al. Bone marrow stromal cells improve cardiac performance in healed infarcted rat hearts. Am J Physiol Heart Circ Physiol. 2004 Aug;287(2).
67. Loffredo FS, Steinhauser ML, Gannon J, Lee RT. Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. Cell Stem Cell. 2011 Apr 8;8(4):389–98.
68. Zhen H, Wang J, Xue L, Rui J, Liu X, Chen Y. LPS-pretreated bone marrow stem cells as potential treatment for myocardial infraction. Front Biosci (Landmark Ed). 2012 Jan 1;17(4):1294–303.
69. Zhong Z, Hu JQ, Wu XD, Sun Y, Jiang J. Myocardin-related transcription factor-A-overexpressing bone marrow stem cells protect cardiomyocytes and alleviate cardiac damage in a rat model of acute myocardial infarction. Int J Mol Med. 2015 Sep 1;36(3):753–9.
70. Li X, Xie X, Yu Z, Chen Y, Qu G, Yu H, et al. Bone marrow mesenchymal stem cells-derived conditioned medium protects cardiomyocytes from hypoxia/reoxygenation-induced injury through Notch2/mTOR/autophagy signaling. J Cell Physiol. 2019 Oct 1;234(10):18906–16.
71. Carr CA, Stuckey DJ, Tatton L, Tyler DJ, Hale SJM, Sweeney D, et al. Bone marrow-derived stromal cells home to and remain in the infarcted rat heart but fail to improve function: an in vivo cine-MRI study. Am J Physiol Heart Circ Physiol. 2008 Aug;295(2).
72. Blume GG, Machado-Junior PAB, Simeoni RB, Bertinato GP, Tonial MS, Nagashima S, et al. Bone-Marrow Stem Cells and Acellular Human Amniotic Membrane in a Rat Model of Heart Failure. Life (Basel). 2021 Sep 1;11(9).
73. Xie DM, Li YL, Li J, Li Q, Lu G, Zhai Y, et al. CD51 distinguishes a subpopulation of bone marrow mesenchymal stem cells with distinct migratory potential: a novel cell-based strategy to treat acute myocardial infarction in mice. Stem Cell Res Ther. 2019 Nov 20;10(1).
74. Omar AM, Meleis AE, Arfa SA, Zahran NM, Mehanna RA. Comparative Study of the Therapeutic Potential of Mesenchymal Stem Cells Derived from Adipose Tissue and Bone Marrow on Acute Myocardial Infarction Model. Oman Med J. 2019 Nov 1;34(6):534–43.

75. Petchdee S, Pattanapon N, Bootcha R, Srivattanakul P, Songserm T. Dental tissue-derived stem cells exerts therapeutic effects on chronic myocardial infarction model of rabbit. Cardiology (Pakistan). 2014;9(1):1–6.
76. Wang JA, Fan YQ, Li CL, He H, Sun Y, Lu BJ. Human bone marrow-derived mesenchymal stem cells transplanted into damaged rabbit heart to improve heart function. J Zhejiang Univ Sci B. 2005 Apr;6(4):242–8.
77. Liu JF, Wang BW, Hung HF, Chang H, Shyu KG. Human mesenchymal stem cells improve myocardial performance in a splenectomized rat model of chronic myocardial infarction. J Formos Med Assoc. 2008;107(2):165–74.
78. Shyu KG, Wang BW, Hung HF, Chang CC, Shih DTB. Mesenchymal stem cells are superior to angiogenic growth factor genes for improving myocardial performance in the mouse model of acute myocardial infarction. J Biomed Sci. 2006 Jan;13(1):47–58.
79. Rasmussen JG, Frøbert O, Holst-Hansen C, Kastrup J, Baandrup U, Zachar V, et al. Comparison of human adipose-derived stem cells and bone marrow-derived stem cells in a myocardial infarction model. Cell Transplant. 2014 Feb;23(2):195–206.
80. Gandia C, Armiñan A, García-Verdugo JM, Lledó E, Ruiz A, Miñana MD, et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells. 2008 Mar 1;26(3):638–45.
81. Isomi M, Sadahiro T, Ieda M. Progress and Challenge of Cardiac Regeneration to Treat Heart Failure. J Cardiol. 2019 Feb 1;73(2):97–101.
82. Mardanpour P, Nayernia K, Khodayari S, Khodayari H, Molcanyi M, Hescheler J. Application of Stem Cell Technologies to Regenerate Injured Myocardium and Improve Cardiac Function. Cell Physiol Biochem. 2019;53(1):101–20.
83. Henning RJ. Stem cells in cardiac repair. Future Cardiol. 2011 Jan;7(1):99–117.
84. Fan M, Chen W, Liu W, Du GQ, Jiang SL, Tian WC, et al. The Effect of Age on the Efficacy of Human Mesenchymal Stem Cell Transplantation after a Myocardial Infarction.
85. Liu Y, Liu T, Han J, Yang Z, Xue X, Jiang H, et al. Advanced age impairs cardioprotective function of mesenchymal stem cell transplantation from patients to myocardially infarcted rats. Cardiology (Switzerland). 2014;128(2):209–19.
86. Song HF, He S, Li SH, Yin WJ, Wu J, Guo J, et al. Aged Human Multipotent Mesenchymal Stromal Cells Can Be Rejuvenated by Neuron-Derived Neurotrophic Factor and Improve Heart Function After Injury. JACC Basic Transl Sci. 2017 Dec 1;2(6):702–16.
87. Yamaguchi S, Shibata R, Yamamoto N, Nishikawa M, Hibi H, Tanigawa T, et al. Dental pulp-derived stem cell conditioned medium reduces cardiac injury following ischemia-reperfusion. Sci Rep. 2015 Nov 6;5.
88. Li X, Xie X, Yu Z, Chen Y, Qu G, Yu H, et al. Bone marrow mesenchymal stem cells-derived conditioned medium protects cardiomyocytes from hypoxia/reoxygenation-induced injury through Notch2/mTOR/autophagy signaling. J Cell Physiol. 2019 Oct 1;234(10):18906–16.
89. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res. 2008 Nov 21;103(11):1204–19.

90. Kubo H, Berretta RM, Jaleel N, Angert D, Houser SR. c-Kit+ bone marrow stem cells differentiate into functional cardiac myocytes. Clin Transl Sci. 2009;2(1):26–32.
91. Bao L, Meng Q, Li Y, Deng S, Yu Z, Liu Z, et al. C-Kit Positive Cardiac Stem Cells and Bone Marrow-Derived Mesenchymal Stem Cells Synergistically Enhance Angiogenesis and Improve Cardiac Function After Myocardial Infarction in a Paracrine Manner. J Card Fail. 2017 May 1;23(5):403–15.
92. Chien KR. Lost and found: cardiac stem cell therapy revisited. Journal of Clinical Investigation. 2006 Jul 7;116(7):1838.
93. Kittivarakarn P, Penna M, Acosta Z, Pelaez D, Montero R, Andreopoulos FM, et al. Cardiomyotic induction and proliferation of dental stem cells on electrospun scaffolds. AIMS Bioengineering 2016 2:139. 2016;3(2):139–55.
94. Dai W, Hale SL, Martin BJ, Kuang JQ, Dow JS, Wold LE, et al. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. Circulation. 2005 Jul 12;112(2):214–23.
95. Di Scipio F, Sprio AE, Folino A, Carere ME, Salamone P, Yang Z, et al. Injured cardiomyocytes promote dental pulp mesenchymal stem cell homing. Biochimica et Biophysica Acta (BBA) - General Subjects. 2014 Jul 1;1840(7):2152–61.
96. Bronckaers A, Hilkens P, Fanton Y, Struys T, Gervois P, Politis C, et al. Angiogenic Properties of Human Dental Pulp Stem Cells. PLoS One. 2013 Aug 7;8(8):e71104.
97. Baru O, Nutu A, Braicu C, Cismaru CA, Berindan-Neagoe I, Buduru S, et al. Angiogenesis in Regenerative Dentistry: Are We Far Enough for Therapy? Int J Mol Sci. 2021 Jan 2;22(2):1–19.
98. Hilkens P, Fanton Y, Martens W, Gervois P, Struys T, Politis C, et al. Pro-angiogenic impact of dental stem cells in vitro and in vivo. Stem Cell Res. 2014 May 1;12(3):778–90.
99. Fazel S, Cimini M, Chen L, Li S, Angoulvant D, Fedak P, et al. Cardioprotective c-kit+ cells are from the bone marrow and regulate the myocardial balance of angiogenic cytokines. Journal of Clinical Investigation. 2006 Jul 7;116(7):1865.
100. Zhou H, Li X, Yin Y, He XT, An Y, Tian BM, et al. The proangiogenic effects of extracellular vesicles secreted by dental pulp stem cells derived from periodontally compromised teeth. Stem Cell Res Ther. 2020 Mar 6;11(1).
101. Yang H, Shin S, Ahn J, Choi Y, Kim KH, Chung CJ. Local injection of pulp cells enhances wound healing during the initial proliferative phase through the stimulation of host angiogenesis. J Endod. 2013 Jun;39(6):788–94.

11. APPENDIX

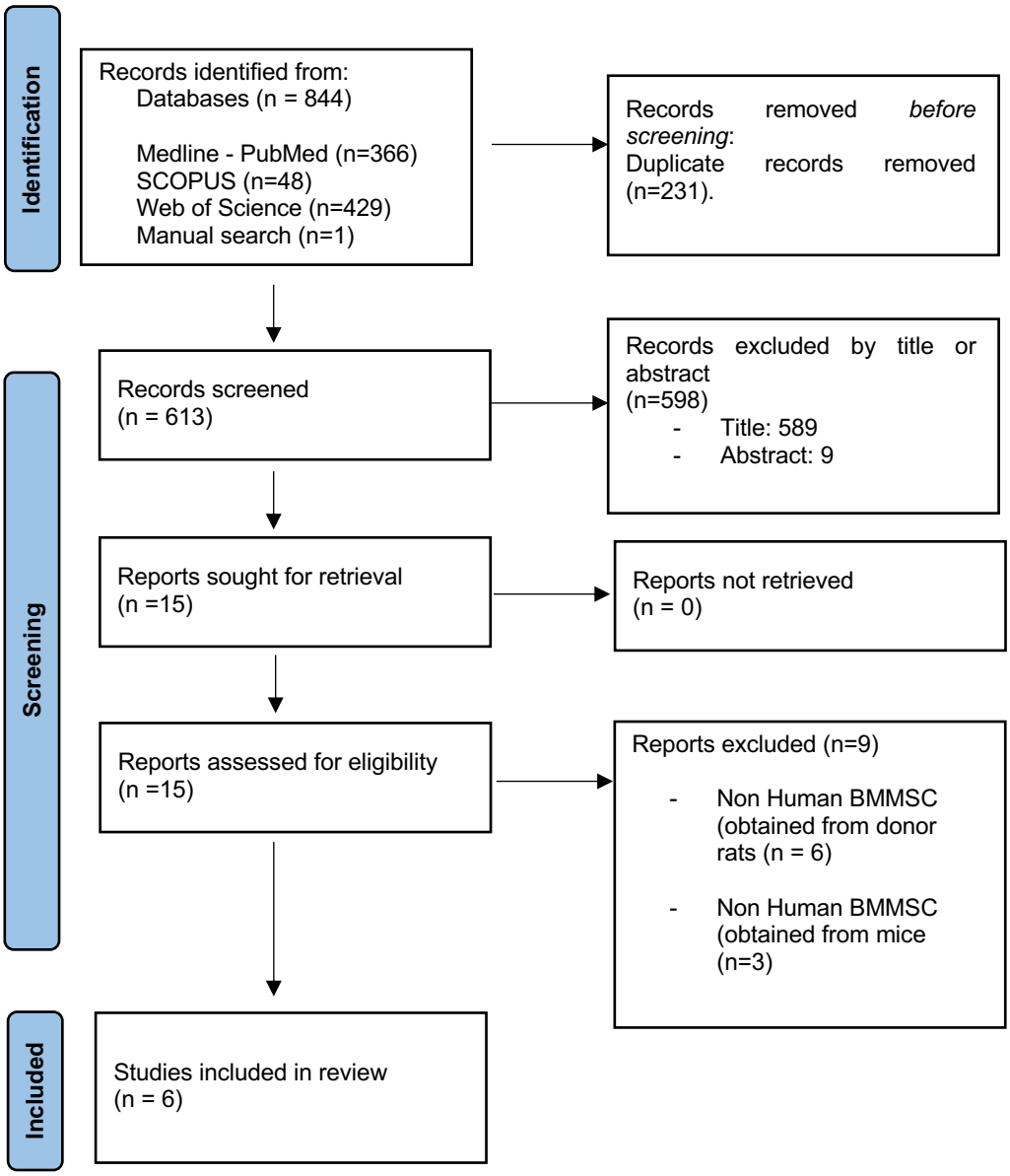

Figure.1. Search Flow diagram and title selection process of our systematic review.

	Soontaree *et al.* 2014	Wang *et al.* 2005	Gandia *et al.* 2008	Liu *et al.* 2008	Shyu *et al.* 2006	Rasmussen *et al.* 2014
1. Study design For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. c. The experimental unit (e.g. a single animal, litter, or cage of animals).	2	2	2	2	2	2
2. Sample size a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	1	1	2	1	2	1
3. Inclusion and exclusion criteria a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	2	2	2	2	2	2
4. Randomization a. State whether randomization was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomization sequence. b. Describe the strategy used to minimize potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	1	1	1	1	1	1
5. Blinding Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	0	0	2	2	2	2
6. Outcome measures a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	2	2	2	2	2	2
7. Statistical methods a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	2	2	2	2	2	2
8. Experimental animals a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	1	1	1	1	1	1
9. Experimental procedures For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatization periods). d. Why (provide rationale for procedures).	1	1	1	1	1	1

10. Results For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	2	2	2	2	2	2
11. Abstract Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	2	2	1	1	1	2
12. Background a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	2	2	2	2	2	2
13. Objectives Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	2	2	2	2	2	2
14. Ethical statement Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	2	2	2	2	2	2
15. Housing and husbandry Provide details of housing and husbandry conditions, including any environmental enrichment.	0	0	0	0	0	0
16. Animal care and monitoring a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	1	1	1	1	1	1
17. Interpretation/ scientific implications a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	2	2	2	2	2	2
18. Generalizability/ translation Comment on whether, and how, the findings of this study are likely to generalize to other species or experimental conditions, including any relevance to human biology (where appropriate).	2	2	2	2	2	2
19.Protocol registration Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	1	1	1	1	1	1
20. Data access Provide a statement describing if and where study data are available.	2	2	2	2	2	2
21. Declaration of interests a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.	2	0	2	2	2	2

b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.						
Coefficient:	32/42 **0,76**	30/42 **0,71**	34/42 **0,81**	33/42 **0,78**	34/42 **0,81**	34/42 **0,76**
Quality:	**Average**	**Average**	**Excellent**	**Average**	**Excellent**	**Average**

Figure.2. Quality coefficients of the studies reviewed according to ARRIVE 2.0 guidelines.

Figure.3. Quality assessment by domain according to ARRIVE 2.0 guidelines.

Table 1. Excluded articles (and reason for exclusion) of our systematic review.

Authors. Year	Publication	Reason for exclusion
Olivares EL. 2004 (66)	Am J Physiol Heart Circ Physiol	Non-Human BMMSC (obtained from donor rats)
Loffredo FS. 2011 (67)	Cell Stem Cell	Non-Human BMMSC (obtained from donor rats)
Zhen H. 2012 (68)	Front Biosci (Landmark Ed)	Non-Human BMMSC (obtained from mice
Zhong Z. 2015 (69)	Int J Mol Med	Non-Human BMMSC (obtained from donor rats)
Li X. 2019 (70)	J Cell Physiol	Non-Human BMMSC (obtained from donor rats)
Carr CA. 2008 (71)	Am J Physiol Heart Circ Physiol	Non-Human BMMSC (obtained from donor rats)
Blume GG. 2021 (72)	Life (Basel)	Non-Human BMMSC (obtained from donor rats)
Xie DM. 2019 (73)	Stem Cell Res Ther	Non-Human BMMSC (obtained from mice
Omar AM. 2019 (74)	Oman Med J	Non-Human BMMSC (obtained from donor rats)

Table 2. Characteristics of the reviewed studies

Authors. Year	Type of study	Type of transplanted cell	Model of MI	Sample size	Administration route	Time elapsed after transplantation
Petchdee *et al.*(75) 2014	Experimental animal study	Human SHED	Rabbit Model	n= 16 (Control group n=8, Cell treated group n=8)	Intravenous injection through rabbit marginal ear vein	4 weeks
Wang *et al.*(76) 2005	Experimental animal study	Human BMMSC	Rabbit Model	n= 25 (Control group n=13, Cell treated group n=12)	Intramyocardial injection into the border area of the ischemic myocardium	4 weeks
Gandia *et al.*(80) 2008	Experimental animal study	Human DPSCs	Rat Model	n= 16 (Control group n=9, Cell treated group n=7)	Intramyocardial transplantation in five injections at five points of the infarct border	4 weeks
Liu *et al.*(77) 2008	Experimental animal study	Human BMMSC	Rat Model	n=23 (Control group n=12, Cell treated group n=11)	Intramyocardial injection at the left anterior free wall	4 weeks
Shyu *et al.*(78) 2006	Experimental animal study	Human BMMSC	Mouse model	n=14 (Control group n=7, Cell treated group n=7)	Intramyocardial injection	4 weeks
Rasmussen *et al.*(79) 2014	Experimental animal study	80 years old Human BMMSC	Rat Model	n=16 (Control group n=7 Cell treated group n=9)	Intramyocardial injection	4 weeks

Table 3. Outcomes synthesis by study

Authors. Year	Type of study	Type of transplanted cell	Model of MI	Sample size	Administration route	Time elapsed after MI induction	Outcomes
Petchdee *et al.*(75) 2014	Experimental animal study	Human SHED	Rabbit Model	n=16	Intravenous injection through rabbit marginal ear vein	4 weeks	1) ❌ Non-significant results in cardiac function 2) ✅ Significant infarct size reduction 3) ✅ Observed angiogenesis
Wang *et al.*(76) 2005	Experimental animal study	Human BMMSC	Rabbit Model	n=25	Intramyocardial injection injecting into the border area of the ischemia myocardium.	4 weeks	1) ✅ Significant improvement in cardiac function 2) ✅ Significant infarct size reduction
Gandia *et al.*(80) 2008	Experimental animal study	Human DPSCs	Rat Model	n=16	Intramyocardial transplantation in five injections at five points of the infarct border	4 weeks	1) ✅ Significant improvement in cardiac function 2) ✅ Significant infarct size reduction 3) ✅ Significant vascular density increase
Liu et al.(77) 2008	Experimental animal study	Human BMMSC	Rat Model	n=23	Intramyocardial injection at the left anterior free wall	4 weeks	1) ✅ Significant improvement in cardiac function 2) ✅ Significant infarct size reduction 3) ✅ Significant vascular density increase
Shyu et al.(78) 2006	Experimental animal study	Human BMMSC	Mouse model	n=14	Intramyocardial injection	4 weeks	1) ✅ Significant improvement in cardiac function 2) ✅ Significant infarct size reduction 3) ✅ Significant vascular density increase
Rasmussen *et al.*(79) 2014	Experimental animal study	80 years old Human BMMSC	Rat Model	n=16	Intramyocardial injection	4 weeks	1) ❌ Non-significant results in cardiac function 2) ❌ Non-significant results in infarct size reduction 3) ❌ Non-significant results in vascular density increase

Table 4. Echocardiographic parameters of LVDd, LVDs and FS by study

DPSCs	**LVDd (mm) Control 4 weeks**	**LVDd (mm) DPSCs 4 weeks**	**LVDs (mm) Control 4 weeks**	**LVDs (mm) DPSCs 4 weeks**	**FS% Control 4 weeks**	**FS% DPSCs 4 weeks**	**FS evolution %**
Gandia *et al.* (80)	7.60 ± 0.18	7.26 ± 0.32	5.63 ± 0.23	4.94 ± 0.24	24.2 ± 1.6	32.9 ± 1.6	+ 36.95 %
Weighted mean DPSCs: + 35.95% FS							
hBMMSC	**LVDd (mm) Control 4 weeks**	**LVDd (mm) hBMMSC 4 weeks**	**LVDs (mm) Control 4 weeks**	**LVDs (mm) hBMMSC 4 weeks**	**FS% Control 4 weeks**	**FS% hBMMSC 4 weeks**	**FS evolution %**
Wang *et al.* (76)	15.4 ± 1.4	11.2 ± 1.8	9.7 ± 0.5	6.7 ± 0.5	37.7 ± 3.28	40.2 ± 3,3	+ 6.68%
Liu *et al.* (77)	7.8 ± 0.2	6.5 ± 0.1	5.4 ± 0.6	3.0 ± 0.1	31.5 ± 6.5	53.5 ± 1.7	+ 69.84%
Shyu *et al.* (78)	4.6 ± 0.2	3.6 ± 0.1	3.1 ± 0.3	2.2 ± 0.1	34.2 ± 2.3	44.4 ± 2.3	+ 30.01%
Rasmussen *et al.* (79)	-	-	-	-	-	-	Non-significant= 0%
Weighted mean hBMMSC: +27.13% FS							
SHED	**LVDd (mm) Control 4 weeks**	**LVDd (mm) SHED 4 weeks**	**LVDs (mm) Control 4 weeks**	**LVDs (mm) SHED 4 weeks**	**FS% Control 4 weeks**	**FS% SHED 4 weeks**	**FS evolution %**
Petchdee *et al*. (75)	-	-	-	-	-	-	Non-significant= 0%

Table 5. Infarct size reduction results

	Infarct size Control group after 4 weeks (mm)	Infarct size Treated group after 4 weeks (mm)	Infarct size evolution %
DPSCs			
Gandia *et al.* (80)	21.2 ± 1.6 %	15.9 ± 1.7 %	-25%
Weighted mean reduction of infarct size: - 25%			
SHED			
Petchdee *et al.* (75)	19.9 ± 0.03 %	10.9 ± 0.02 %	-45.2%
Weighted mean reduction of infarct size: - 45.2%			
hBMMSC			
Liu *et al.* (77)	54.9±3.3%	35.4±3.4%	-35.5%
Rasmussen *et al.* (79)	-	-	Non-significant = 0%
Shyu *et al.* (78)	66.1±1.5%	58.1±1.1%	-12.1%
Weighted mean reduction of infarct size: -18.67%			

Table 6. Descriptive results of vascular density evolution

	Control group vascular density after 4 weeks number of vessels per unit area (mm^2)	Treated group vascular density after 4 weeks number of vessels per unit area (mm^2)	Vascular density evolution %
DPSCs			
Gandia *et al.* (80)	518 ± 115/mm^2	868 ± 85/mm^2	+ 67.51%
Weighted mean increase of vascular density: + 67.51%			
SHED			
Petchdee *et al.* (75)	-	-	Increased number of capillaries based on H&E staining but not quantified.
Weighted mean increase of vascular density: Not quantified			
hBMMSC			
Liu *et al.* (77)	144 ± 16/mm^2	938±42/mm^2	+84.64%
Rasmussen *et al.* (79)	-	-	Non-significant = 0%
Shyu *et al.* (78)	439±27/mm^2	1119±17/mm^2	+60.8%
Weighted mean increase of vascular density: + 34.57%			

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Front page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1,3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	17-18
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	21
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	24
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	25-26
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	25-26
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	27
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	27
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	27-28
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	27-28
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	28-29
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	28-29
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	27,29

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	27,29
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	27,29
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	29
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	32,34
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	32-33
Study characteristics	17	Cite each included study and present its characteristics.	33-34
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	35,37
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	66-68
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	38,42
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	44,47
	23b	Discuss any limitations of the evidence included in the review.	48
	23c	Discuss any limitations of the review processes used.	48
	23d	Discuss implications of the results for practice, policy, and future research.	49
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

THERAPEUTIC POTENTIAL OF HUMAN DENTAL PULP STEM CELLS IN CARDIAC REPAIR, A NEW HOPE FOR INTERDISCIPLINARY REGENERATIVE MEDICINE? – A SYSTEMATIC REVIEW

Running title: Therapeutic potential of human dental pulp stem cells in cardiac repair, a new hope for interdisciplinary regenerative medicine?

Authors:

Emilie Caulet [1], María del Carmen Carceller Zazo [1]

[1] *Universidad Europea de Valencia. Faculty of Health Sciences. Department of Dentistry*

Corresponding and reprints author:

María del Carmen Carceller Zazo
Paseo Alameda 7, Valencia
46010, Valencia

carmen.carceller@universidadeuropea.es

Abstract :

Introduction: Over the past 2 decades, mesenchymal stem cells (MSCs) have become the basis of the emerging field of regenerative medicine due to their self-renewal and multilineage differentiation abilities. Despite treatment advancements, myocardial infarction remains a significant cause of mortality and disabilities. Dental pulp stem cells (DPSCs) offer promising regenerative properties for cardiac regeneration.

Aims: This review aims to assess the improvement achieved by human DPSCs and stem cells from human exfoliated deciduous teeth (SHED) in cardiac function, infarct size reduction, and angiogenesis compared to human bone marrow MSCs (hBMMSC) in rodent models of myocardial infarction.

Material and Methods: An electronic search was conducted in PubMed, Scopus and Web of Science databases on cardiac regeneration achieved by DPSCs, SHED or hBMMSC in rodent models of myocardial infarction. The search has been completed by manual search. The limitation date was January 2023.

Results: Above 844 studies identified through the search process, 6 studies were included for complying with the inclusion criteria: 1 study describes the transplantation of SHED, 4 describe the transplantation of hBMMSC, and 1 describes the transplantation of DPSCs. Cardiac regeneration has been evaluated through 3 parameters: Cardiac function improvement (through fractional shortening (FS) evolution), infarct size reduction and angiogenesis. Regarding FS improvement: DPSCs showed a mean increase of 36.95%, hBMMSC 27.13%, and SHED did not provide any significant result. Infarct size was reduced by 25% with DPSCs, 45.2% by SHED and 18.67% by hBMMSC. Angiogenesis was observed in all groups but not quantified in the SHED group, the DPSCs group presented a mean increase of vascular density of 67.51% and the hBMMSC group 34.57%.

Conclusion: Despite limitations, dental pulp stem cells seemed to achieve greater improvement in cardiac function, reduction of the infarct size as well as induction of angiogenesis compared to hBMMSC in rodent models of myocardial infarction.

Keywords: *Human dental pulp stem cells, Dental pulp stem cells isolated from the pulp of human exfoliated deciduous teeth, Human bone marrow-derived mesenchymal stem cells, Cardiac repair.*

INTRODUCTION:

In a world of constant evolution dentistry has recently been embossing its presence through major leaps in interdisciplinary medical research. Dental professionals are consistently seeking to improve patient treatment. Over the past 2 decades, mesenchymal stem cells (MSCs) have become the basis of the emerging field of regenerative medicine thanks to their abilities of self-renewal and multilineage differentiation (1). Although the regenerative capacity of human dental tissue is limited, the discovery of permanent dentition dental pulp stem cells (DPSCs) as well as dental pulp stem cells isolated from the pulp of human exfoliated deciduous teeth (SHED) in the early 2000s, has opened new and surprising outline in tooth regeneration as well as non-dental tissue regeneration leading the way to interdisciplinary collaboration (2,3). Despite recent substantial advances in prevention and treatment, myocardial infarction (MI) and cardiovascular diseases remain a leading cause of death with a prevalence of 3 million people worldwide annually causing irreversible damage to cardiomyocytes due to oxygen supply discontinuation (4). The lack of regenerative capacity of the myocardium highlights the importance of reducing cardiomyocyte loss, stimulating neovascularization, and maintaining cardiac function. Thus, dental stem cell transplantation appears to be a solid option in the field of regenerative medicine and currently delivers promising results in cardiac regeneration. While present studies are mostly conducted with human bone marrow-derived mesenchymal stem cells (hBMMSC), DPSCs and SHED showed higher proliferation rate compared to hBMMSC as well as a differentiating potential into myocytes, melanocytes, active neurons, hepatic-like cells, insulin-producing cells and cardiomyocytes (5–11). Mesenchymal stem cell therapy and heart regeneration are mainly based on indirect (paracrine signaling) and direct (trans-differentiation) mechanisms as well as neovascularization, immunomodulation, and cardiac remodeling (12–14).

In this systematic review we aim to compare the therapeutic potential of human dental pulp stem cells (DPSCs & SHED) versus hBMMSC in myocardial repair after myocardial infarction in rodents. Primarily the cardiac function has been evaluated through comparison of fractional shortening evolution with DPSCs or SHED transplantation

compared to hBMMSC transplantation. Secondly, the reduction of the infarct size and angiogenesis have been compared between groups.

MATERIAL AND METHODS:

The present systematic review was conducted following the PRISMA statement (2020) (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guideline statement (15).

-Focus question: The focus question was set according to the structured PICO question. The question format was set as follows:

- **P** (Population): Rodent models of myocardial infarction
- **I** (Intervention): DPSCs or SHED transplantation
- **C** (Comparison): hBMMSC transplantation
- **O** (Outcomes):
  - O1: Improvement of cardiac function
  - O2: Reduction of the infarct size
  - O3: Induction of angiogenesis

-Eligibility criteria:

The Inclusion criteria were:

- Study design: Experimental animal studies ethically approved; studies published in English, French or Spanish up to January 2023.
- Population: *In vivo* rodent models of myocardial infarction. Species: Rats, Mice, Rabbits. Sex: either male or female rodent.
- Intervention: Transplantation of DPSCs or SHED or hBMMSC.
- Outcomes: Studies measuring improvement of cardiac function as the primary outcome, reduction of the infarct size and angiogenesis induction as secondary outcomes.

The exclusion criteria were peer reviews, reviews, letters to the editor or comments, expert opinion, editorials, randomized clinical trials, case series, studies on humans. In

addition to this were excluded all studies conducted with animal stem cells. There was no restriction applied about the publication date.

- **Information sources and data search**:

A search was carried out in the three following databases: PubMed, Scopus and Web of Science using the following keywords: “myocardial infarction”, “rodentia”, “acute myocardial infarction”, “chronic myocardial infarction”, “rodents”, “myocardial ischemia”, “reperfusion”, “human dental pulp stem cells”, “DPSC”, “dental stem cell therapy”, “stem cells from human exfoliated deciduous teeth”, “human Bone marrow-derived mesenchymal stem cells”, “BM-MSC”, “BMSC”, “human bone marrow multipotent mesenchymal stem cells”, “hMSCs", “cardiomyocytic differentiation” “cardiac repair”, “ventricular function”, “myocardial performance”, “cardiac function”.
The keywords were combined with the Boolean operators AND and OR, as well as controlled termed (“MESH” for PubMed) in order to get the best and broadest search results.
The following search strategy in Pubmed was carried out: (((((((("Myocardial infarction" [MeSH Terms]) AND ("Rodentia"[MeSH Terms])) OR (Acute myocardial infarction)) OR (Chronic myocardial infarction)) AND (rodents) OR (myocardial ischemia))) AND ((((("Human dental pulp stem cells") OR ("DPSC")) OR (dental stem cell therapy)) OR ("Stem cells from human exfoliated deciduous teeth"))) OR (((((“Human Bone marrow-derived mesenchymal stem cells") OR ("BM-MSC")) OR ("BMSC")) OR (human bone marrow multipotent mesenchymal stem cells)) OR ("hMSCs"))) AND (((((“Cardiomyocytic differentiation") OR (cardiac repair)) OR ("ventricular function")) OR ("myocardial performance") OR ("cardiac function")) OR ("reperfusion")).
A cross-search of possibly insightful publications was carried out for analysis. The authors of the papers were approached in order to acquire those that were not in full-text databases. The review was cleared of duplicate studies. The search was finished with a check of the references listed in the bibliography of each study in order to find any suitable research that the initial search may have overlooked. The Journal of the American College of Cardiology and Medwell Journals publications were searched manually for scientific literature.

- **Search strategy**:

A three-phase selection process was carried out by two reviewers (EC, CC). Titles were analyzed in the preliminary stage, and irrelevant publications were discarded. The second phase was centered on abstracts, screening was performed, and the selection based itself on the type of study, type of stem cell transplants, type of model of myocardial infarction, and outcome variables. The last phase examined the full-text and data were extracted using a data collection form previously prepared to confirm the eligibility of the studies. Disagreements between reviewers, at each of the stages, were resolved by discussion and, where necessary, a third reviewer was consulted. The degree of agreement regarding the inclusion of potential studies was calculated by Cohen's kappa test (k-statistics) for the second and third stages of selection.

- **Data extraction**:

Information was extracted from studies and organized into tables according to authors with the year of publication, type of study, the type of stem cell transplantation (DPSCs, SHED or hBMMSC), type of model of myocardial injury (rats, mouse, rabbits), size of the sample, type of administration route (intravenous or intramyocardial), time elapsed after transplantation (weeks), outcomes (improvement of cardiac function, infarct size reduction, vascular density evolution). Echocardiographic parameters such as left ventricular internal dimension at end-diastole (LVDd) and end-systole (LVDs) in millimeters, as well as fractional shortening (FS) in percentages, were analyzed 4 weeks after transplantation and compared to control groups. When fractional shortening was not provided it was calculated according to the following formula: FS= [[(LVDd - LVDs)/LVDd]*100] (16). Cross-sections of excised hearts were stained and infarct size extension (infarct area/total left ventricular area) was expressed as a percentage of the total left ventricular area as a mean of all slices from each heart. Results were analyzed in percentages and compared to the control group. Vascular density was evaluated by immunostaining. The number of vessels per unit area (mm^2) was then expressed as percentages of change and compared to control groups.

- **Quality and risk of bias assessment**:

The quality assessment of experimental animal studies was based on the ARRIVE 2.0 (Animal Research: Reporting *in vivo* Experiment) guidelines and performed by two reviewers (EC,CC) (17). Articles were examined, and bias was evaluated according to the evaluation of the 21 items (10 from the “Essential 10” and 11 from the “Recommended set”): if a publication satisfied all the subitems, it was scored as "reported" and given 2 points. If a publication did not satisfy any of the subitems, it was scored as "not reported" and given 0 points. If the details provided for the subitems were unclear, it was scored as "unclear" and given 1 point. In this way, the study's quality was determined by applying a pre-established coefficient, which ranged from 0.8 to 1 for excellent quality, 0.5 to 0.8 for average quality, and less than 0.5 for poor quality. The coefficient was calculated by adding up the total points earned by each study and dividing it by the maximum possible points per study, which was 42. Quality assessment by domain has also been evaluated. The degree of inter-examiner agreement in the assessment of methodological quality was obtained with Cohen's kappa test, following the scale proposed by Landis and Koch (18).

- **Data synthesis**:

A descriptive analysis of the outcome variables was performed. Since the means calculated in the studies analyzed came from different samples size of rodents it was necessary to calculate the weighted mean with the objective of obtaining more accurate results. The means of values of primary variables were grouped by research group (DPSCs, SHED and hBMMSC). Due to the lack of randomized studies comparing all treatment groups, a meta-analysis was not possible, hence the results were concentrated on a descriptive analysis of the factors.

RESULTS:

- **Study selection**:

A total of 843 studies were identified through the initial search process: Medline-PubMed (n=366), SCOPUS (n=48) and Web of Science (n=429). An additional study was obtained through manual search (reference list and primary sources). Of these

publications 24 were identified as potentially eligible by screening of titles. Screening of abstracts of these 24 publications lead to the selection of 15 articles. Finally, full-text articles were exanimated leading to the final selection of the 6 articles included in the present systematic review (Figure. 1). The k-value for inter-examiner agreement on the inclusion of studies was 0.87 (titles and abstracts) and 1.0 (full texts) indicating "good" and "complete" agreement, respectively, according to Landis and Koch's criteria (18).

- **Study characteristics**:

Of the 6 articles selected in the present review, 1 describes the transplantation of SHED (19), 4 describe the transplantation of hBMMSC (20–23) and 1 describes the transplantation of DPSCs (24). All of them are animal experimental studies. A total of 110 rodents were induced with myocardial infarction, of which 54 rodents were treated with human mesenchymal stem cells including 8 rodents treated with SHED, 7 rodents treated with DPSCs and 39 were treated with hBMMSC. Two studies used a rabbit model of myocardial infarction (19,20), 3 used a rat model (21,23,24) and 1 used a mouse model (22). Regarding the administration route of mesenchymal stem cells, 5 studies used intramyocardial injection (20–24), 1 used and an intravenous injection (19). All data were collected 4 weeks after transplantation (Table 1).

- **Risk of bias**:

In this review, two studies were rated as having excellent quality (22,24), while the remaining four studies were classified as having average quality (19–21,23). The item that posed the highest risk of bias was housing and husbandry, as indicated in Figure 3. The k-value (Cohen kappa test) for inter-reviewer agreement on methodological quality was 0.9 according to the Landis & Koch scale (18).

- **Synthesis of results**:

<u>Cardiac function improvement:</u>

The studies conducted by Petchdee *et al.* (19) with SHED and Rasmussen *et al.* (23) with hBMMSC did not provide significant results in FS evolution between the control group and treated groups. Gandia *et al*. (24) observed an increase in FS of 36.95% compared to the control group 4 weeks after intramyocardial injection of DPSCs at the infarct

border. The control group presented a significant deterioration in cardiac function and internal ventricular dimensions. The weighted mean increase of FS in the hBMMSC group was 27.13% compared to the control group 4 weeks after intramyocardial injections (20–23). The highest FS increase was obtained by Liu *et al.* (21) with 69.84%. The lowest has been obtained by Wang *et al.* (20) with an increase of 6.68%. Control groups presented a significant decrease in cardiac function and ventricular dimensions. The only study that did not obtain results of cardiac function improvement with hBMMSC was the one conducted by Rasmussen *et al.* (23). SHED on the other hand did not show a significant improvement in cardiac function (19). The highest improvement in cardiac function was obtained with DPSCs (24) with a significant difference (p=0,010) compared to hBMMSC. Descriptive results on echocardiographic parameters are shown in Table 2.

Reduction of the infarct size

All three types of stem cell treatment: DPSCs, SHED and hBMMSC showed significant results (p<0,5) in the reduction of the infarct size. In the study by Gandia *et al.* (24) DPSCs showed an infarct size reduction of 25% compared to the control group 4 weeks after intramyocardial injection at the border of the infarct. Petchdee *et al.* (19) observed a 45.2% reduction in the infarct size with the intravenous injection of SHED. Regarding the hBMMSC group, Rasmussen *et al.*(23) conducted the only study within this group that did not achieve a significant reduction in infarct size. The weighted mean reduction with hBMMSC was 18.67%. The greatest reduction in infarct size was achieved by SHED with a reduction of 45.2%, followed by the DPSCs group with a reduction of 25% and the least reduction was achieved by hBMMSC group with 18.67%. Descriptive results on infarct size reduction are shown in Table 3.

Induction of angiogenesis

DPSCs induced angiogenesis, resulting in the formation of functional rat-derived blood vessels. There was a 67.51% increase in vascular density compared to the control group (24). Petchdee *et al.* (19) observed an increase in vascular density through histological sections stained with Hematoxylin & Eosin (H&E). A quantitative comparison was not possible as exact values were not provided. The weighted mean increase for the

hBMMSC group was 34.57%, with the highest increase of 84.64% obtained by Liu *et al.* (21).

Once again, the study conducted by Rasmussen *et al.* (23) did not obtain significant results in the induction of angiogenesis. Descriptive results of vascular density evolution are presented in Table 4.

DISCUSSION:

Cardiac function improvement:

The results of this systematic review based on six scientific investigations revealed a greater improvement in cardiac function with DPSCs intramyocardial injection compared to hBMMSC and SHED. The lack of results in cardiac regeneration observed by Rasmussen *et al.* (23) following hBMMSC transplantation may be attributed to the fact that the stem cells used in the experiment were derived from an 80-year-old donor. Indeed, Fan *et al.*(25) et al and Liu *et al*. (26) have discussed the importance of advanced age on the cardioprotective and regenerative capacities of mesenchymal stem cells. Both studies showed that age had a substantial impact on the ability of hBMMSCs to regenerate. Young hBMMSC transplants enhanced functional outcomes following a MI by stopping matrix breakdown and encouraging angiogenesis (25,26). In 2017, Song *et al.* (27) conducted a study where they further explored the rejuvenation of hBMMSC and observed its effectiveness. They found out that cardiac performance improved after myocardial infarction was obtained in mice hearts by transplanting neuron-derived neurotrophic factor over-expressing aged hBMMSC, which enhanced the survival of the implanted stem cell. Regarding SHED although they did not provide significant results in improving cardiac function, they validated the safety of SHED transplantation after myocardial infarction in a rabbit model (19). A study conducted by Yamaguchi *et al.* (28) provided the first evidence that SHED-conditioned medium conferred resistance to acute ischemic damage in the heart: as a matter of fact, SHED-conditioned medium significantly increased ventricular FS, suppressed apoptosis and inflammation in the ischemic heart of mice 7 days after myocardial infarct induction.

Reduction of the infarct size:

The reduction can be explained through ultrastructural examination of the left ventricular wall and peri-infarct zones, which showed an increase in cardiomyocyte bundles that decreased the infarct size and encouraged a higher fraction of myofibroblast. Myofibroblasts are linked to the recovery of ischemic wounds, and their ontogenesis is connected to the morphological alterations seen in fibroblasts under stress-strain (19,24). While DPSCs did not differentiate into cardiomyocytes, engrafted hBMMSC presented cardiomyocytic differentiation conferring them the ability to survive long-term in cardiac tissue (20–22,24). An *in vitro* study conducted by Kittivarakam *et al*. (29) showed that SHED could differentiate into functional cardiomyocytes and were able to proliferate on electrospun scaffolds. We can also add that through longer periods of time allogenic bone marrow mesenchymal stem cells required between 3 to 6 months to express muscle markers, thus not ruling out the possibility that longer periods of transplantation may be necessary to induce the expression of cardiac markers in transplanted DPSCs (30). Donor's age impaired the ability of hBMMSC to reduce the infarct size: Rasmussen *et al.* (23) did not observe any result using the hBMMSC from the 80-year-old donor.

Induction of angiogenesis:

In this review we observed that DPSCs achieved a greater angiogenesis induction with a higher percentage of vascular density increase compared to hBMMSC. Angiogenesis triggered by SHED was not compared because the study did not provide quantitative data. Shyu *et al.* (22) demonstrated that hBMMSC presented a superior potential in enhancing angiogenesis than VEGF and angiogenic growth factors after myocardial infarction in mice. The age of the donor of hBMMSC also played a role in the induction of angiogenesis: Rasmussen *et al.* (23) observed that 80-year-old hBMMSC did not provide any significative result in angiogenesis induction. Fan *et al.* (25), on the other hand, observed that vascular endothelial growth factor (VEGF) and protein levels were significantly elevated in the myocardium after young hBMMSC were transplanted.

Limitations:

The present review revealed a lack of experimental animal studies focused on dental pulp stem cells as only one study is at the present moment focused on DPSCs and myocardial infarction (24) and there is only one similar study focused on SHED (19). For this reason, the results of the present systematic review must be interpreted with caution. Another limitation is the lack of uniformity in the data collected about cardiac function measurement: studies about dental stem cells lacked data about ejection fraction figures and fractional shortening was not always directly measured. Despite this, data about LVDd and LVDs allowed us to calculate FS and thus compare improvement achieved by each group of cells (19,24). A more complete analysis of cardiac function would have required ejection fraction measurements. Similarly, there is a lack of uniformity in the way of measuring capillary density. In some studies, it was obtained through immunostaining, whereas in others was assessed from photomicrographs by computerized image analysis. The age of donor cells also influenced the results and drastically lowered the weighted mean results of the hBMMSC group. The lack of information about the age of donors could not allow us to differentiate results inside the hBMMSC group (23,25–27). Another limitation was the lack of follow-up time to be able to detect potential oncogenic side effects or DPSCs cardiomyocytic differentiation.

Clinical implications and future perspectives

Dental pulp stem cells represent a substantial alternative for non-dental regeneration and treatment of various inflammatory diseases thanks to their immunomodulatory properties and differentiation potential. Pre-clinical implications in experimental animal studies have provided successful promising results opening the way to further research encouraging clinical trials to validate the safety and efficacy of dental pulp stem cell transplantation. In the future, dental stem cells may also be combined with conditioned medium, other type of stem cells, scaffolds and growth factors that may improve their properties in myocardial regeneration. Current improvements in dental pulp stem cell banking along with their abundance, easy accessibility, low tumorigenicity, ethical acceptability make the humble tooth as a strong contender for inter-regenerative medicine.

REFERENCES:

1. Aly LAA. Stem cells: Sources, and regenerative therapies in dental research and practice. World J Stem Cells. 2015;7(7):1047–53.
2. Yamada Y, Nakamura-Yamada S, Kusano K, Baba S. Clinical potential and current progress of dental pulp stem cells for various systemic diseases in regenerative medicine: A concise review. Int J Mol Sci .; 2019; 20(5):1132.
3. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA. 2000; 97(25):13625-30.
4. Kim SJ. Global awareness of myocardial infarction symptoms in general population. Vol. 51, Korean Circ J .2021; 51(12):997-1000.
5. Patil R, Kumar BM, Lee WJ, Jeon RH, Jang SJ, Lee YM, et al. Multilineage potential and proteomic profiling of human dental stem cells derived from a single donor. Exp Cell Res. 2014 ;320(1):92–107.
6. Ishkitiev N, Yaegaki K, Calenic B, Nakahara T, Ishikawa H, Mitiev V, et al. Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. J Endod. 2010;36(3):469–74.
7. Armiñán A, Gandía C, Bartual M, García-Verdugo JM, Lledó E, Mirabet V, et al. Cardiac differentiation is driven by NKX2.5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. Stem Cells Dev. 2009 ;18(6):907–17.
8. Heng BC, Lim LW, Wu W, Zhang C. An Overview of Protocols for the Neural Induction of Dental and Oral Stem Cells In Vitro. Tissue Eng Part B Rev. 2016 ;22(3):220–50.
9. Saghiri MA, Asatourian A, Sorenson CM, Sheibani N. Role of angiogenesis in endodontics: contributions of stem cells and proangiogenic and antiangiogenic factors to dental pulp regeneration. J Endod. 2015 ;41(6):797–803.
10. Stevens A, Zuliani T, Olejnik C, LeRoy H, Obriot H, Kerr-Conte J, et al. Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev. 2008;17(6):1175–84.
11. Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endod. 2009 ;35(11):1536–42.

12. Redgrave RE, Tual-Chalot S, Davison BJ, Singh E, Hall D, Amirrasouli MM, et al. Cardiosphere-Derived Cells Require Endoglin for Paracrine-Mediated Angiogenesis. Stem Cell Reports. 2017 ;8(5):1287.
13. Chimenti I, Smith RR, Li TS, Gerstenblith G, Messina E, Giacomello A, et al. Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice. Circ Res. 2010;106(5):971–80.
14. Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. Cell Mol Life Sci. 2017 ;74(13):2345–60.
15. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
16. Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ, et al.. New Index of Combined Systolic and Diastolic Myocardial Performance: A Simple and Reproducible Measure of Cardiac Function-A Study in Normals and Dilated Cardiomyopathy. J Cardiol. 1995;26(6):357–66.
17. Percie Du Sertid N, Hurst V, Ahluwaliaid A, Alamid S, Aveyid MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Br J Pharmacol. 2020; 177(16):3617-24.
18. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics. 1977 ;33(2):363–74.
19. Petchdee S, Pattanapon N, Bootcha R, Srivattanakul P, Songserm T. Dental tissue-derived stem cells exerts therapeutic effects on chronic myocardial infarction model of rabbit. The Cardiology. 2014;9:1–6.
20. Wang JA, Fan YQ, Li CL, He H, Sun Y, Lu BJ. Human bone marrow-derived mesenchymal stem cells transplanted into damaged rabbit heart to improve heart function. J Zhejiang Univ Sci B. 2005 ;6(4):242–8.
21. Liu JF, Wang BW, Hung HF, Chang H, Shyu KG. Human mesenchymal stem cells improve myocardial performance in a splenectomized rat model of chronic myocardial infarction. J Formos Med Assoc. 2008;107(2):165–74.
22. Shyu KG, Wang BW, Hung HF, Chang CC, Shih DTB. Mesenchymal stem cells are superior to angiogenic growth factor genes for improving myocardial performance in the mouse model of acute myocardial infarction. J Biomed Sci. 2006 ;13(1):47–58.

23. Rasmussen JG, Frøbert O, Holst-Hansen C, Kastrup J, Baandrup U, Zachar V, et al. Comparison of human adipose-derived stem cells and bone marrow-derived stem cells in a myocardial infarction model. Cell Transplant. 2014 ;23(2):195–206.
24. Gandia C, Armiñan A, García-Verdugo JM, Lledó E, Ruiz A, Miñana MD, et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells. 2008 ;26(3):638–45.
25. Fan M, Chen W, Liu W, Du GQ, Jiang SL, Tian WC, et al. The Effect of Age on the Efficacy of Human Mesenchymal Stem Cell Transplantation after a Myocardial Infarction. Rejuvenation Res. 2010; 13(4):429-38.
26. Liu Y, Liu T, Han J, Yang Z, Xue X, Jiang H, et al. Advanced age impairs cardioprotective function of mesenchymal stem cell transplantation from patients to myocardially infarcted rats. Cardiology . 2014;128(2):209–19.
27. Song HF, He S, Li SH, Yin WJ, Wu J, Guo J, et al. Aged Human Multipotent Mesenchymal Stromal Cells Can Be Rejuvenated by Neuron-Derived Neurotrophic Factor and Improve Heart Function After Injury. JACC Basic Transl Sci. 2017 ;2(6):702–16.
28. Yamaguchi S, Shibata R, Yamamoto N, Nishikawa M, Hibi H, Tanigawa T, et al. Dental pulp-derived stem cell conditioned medium reduces cardiac injury following ischemia-reperfusion. Sci Rep. 2015 ;5:16295.
29. Kittivarakarn P, Penna M, Acosta Z, Pelaez D, Montero R, Andreopoulos FM, et al. Cardiomyotic induction and proliferation of dental stem cells on electrospun scaffolds. AIMS Bioengineering. . 2016;3(2):139–55.
30. Dai W, Hale SL, Martin BJ, Kuang JQ, Dow JS, Wold LE, et al. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. Circulation. 2005 ;112(2):214–23.

Funding: *None declared.*

Conflict of interest: *None declared.*

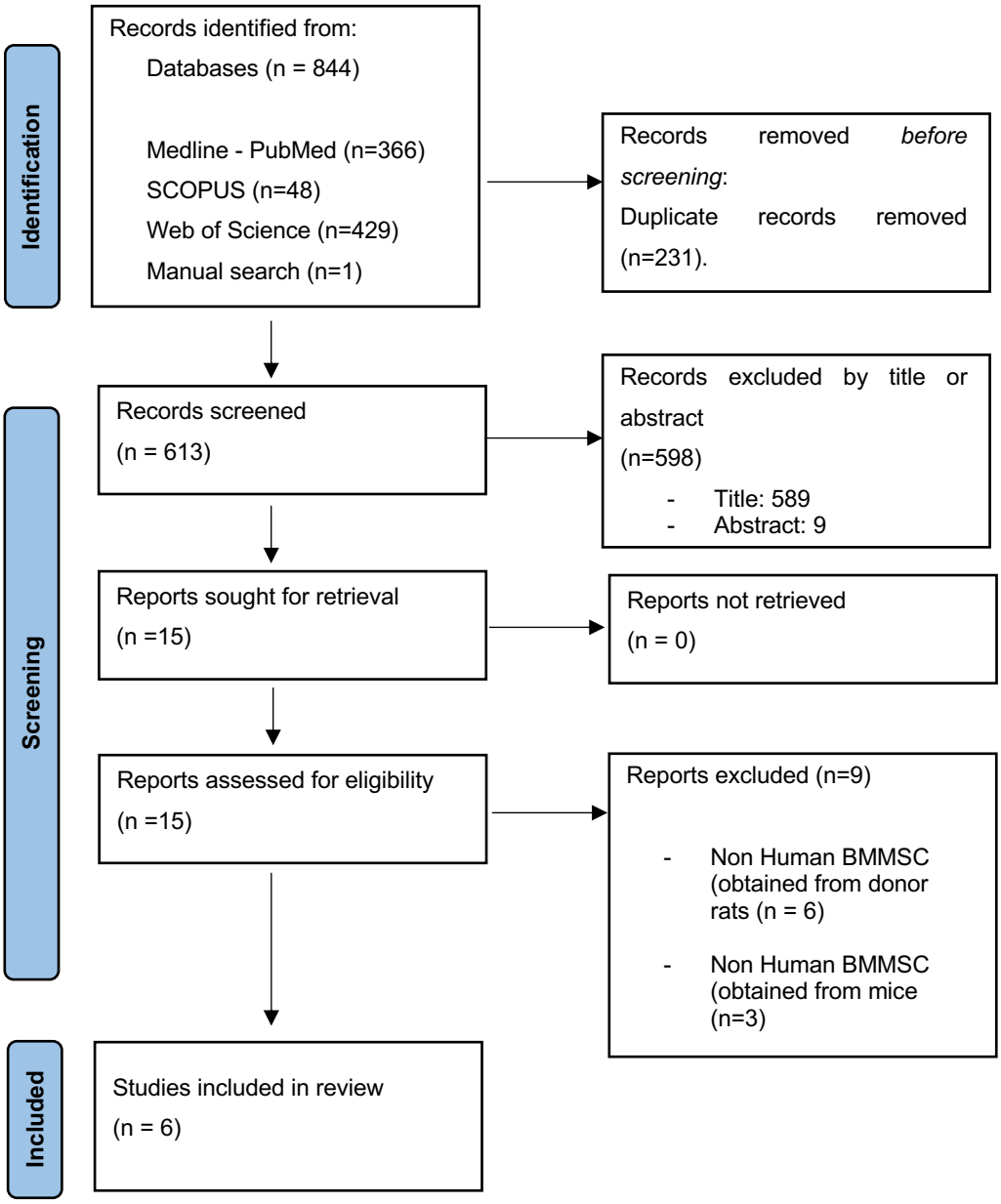

Figure.1. Search Flow diagram and title selection process of our systematic review.

	Soontaree *et al.* 2014	Wang *et al.* 2005	Gandia *et al.* 2008	Liu *et al.* 2008	Shyu *et al.* 2006	Rasmussen *et al.* 2014
1. Study design For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	2	2	2	2	2	2
2. Sample size a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	1	1	2	1	2	1
3. Inclusion and exclusion criteria a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	2	2	2	2	2	2
4. Randomization a. State whether randomization was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomization sequence. b. Describe the strategy used to minimize potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	1	1	1	1	1	1
5. Blinding Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	0	0	2	2	2	2
6. Outcome measures a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	2	2	2	2	2	2
7. Statistical methods a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	2	2	2	2	2	2
8. Experimental animals a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	1	1	1	1	1	1

9. Experimental procedures For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatization periods). d. Why (provide rationale for procedures).	1	1	1	1	1	1
10. Results For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	2	2	2	2	2	2
11. Abstract Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	2	2	1	1	1	2
12. Background a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	2	2	2	2	2	2
13. Objectives Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	2	2	2	2	2	2
14. Ethical statement Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	2	2	2	2	2	2
15. Housing and husbandry Provide details of housing and husbandry conditions, including any environmental enrichment.	0	0	0	0	0	0
16. Animal care and monitoring a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	1	1	1	1	1	1
17. Interpretation/ scientific implications a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	2	2	2	2	2	2
18. Generalizability/ translation Comment on whether, and how, the findings of this study are likely to generalize to other species or experimental conditions, including any relevance to human biology (where appropriate).	2	2	2	2	2	2

19.Protocol registration Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	1	1	1	1	1	1
20. Data access Provide a statement describing if and where study data are available.	2	2	2	2	2	2
21. Declaration of interests a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	2	0	2	2	2	2
Coefficient:	32/42 **0,76**	30/42 **0,71**	34/42 **0,81**	33/42 **0,78**	34/42 **0,81**	34/42 **0,76**
Quality:	**Average**	**Average**	**Excellent**	**Average**	**Excellent**	**Average**

Figure.2. Quality coefficients of the studies reviewed according to ARRIVE 2.0 guidelines.

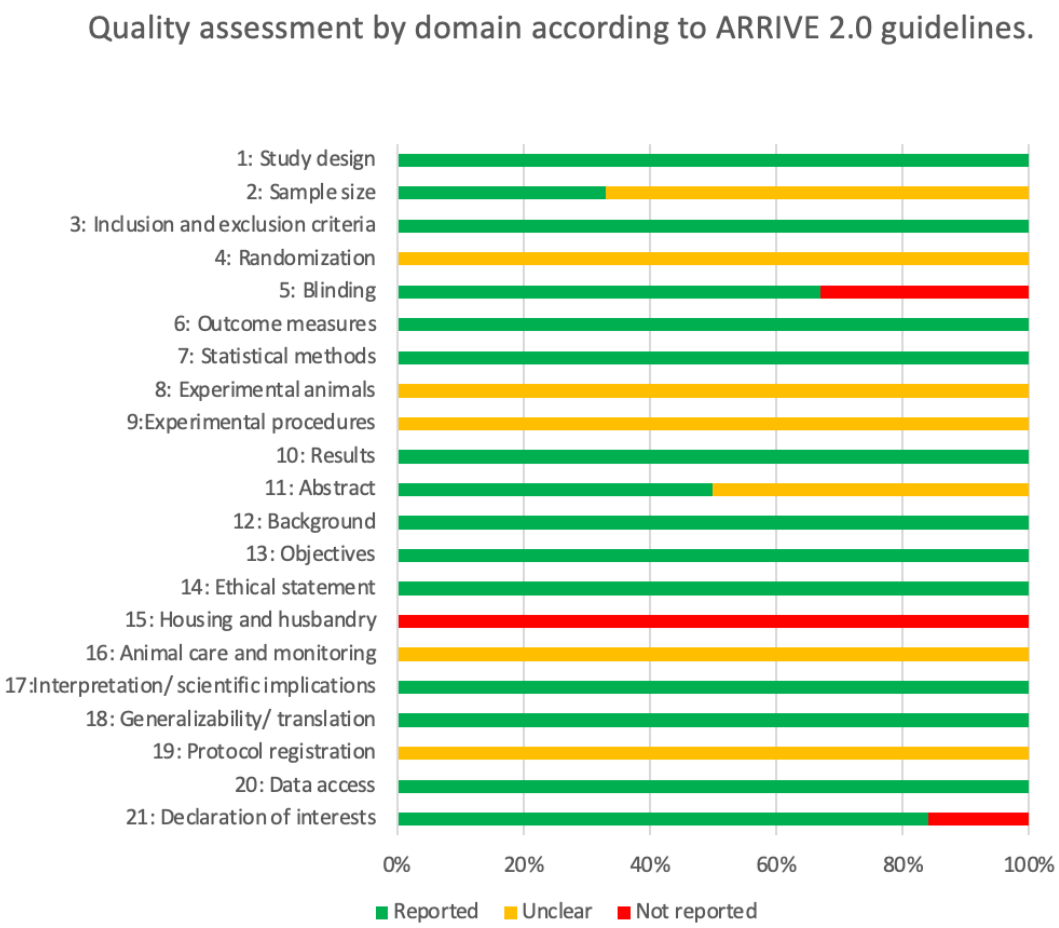

Figure.3. Quality assessment by domain according to ARRIVE 2.0 guidelines.

Table 1. Characteristics of the reviewed studies

Authors. Year	Type of study	Type of transplanted cell	Model of MI	Sample size	Administration route	Time elapsed after MI induction	Outcomes
Petchdee *et al.*(19) 2014	Experimental animal study	Human SHED	Rabbit Model	n=16	Intravenous injection through rabbit marginal ear vein	4 weeks	1) ❌ Non-significant results in cardiac function 2) ✅ Significant infarct size reduction 3) ✅ Observed angiogenesis
Wang *et al.*(20) 2005	Experimental animal study	Human BMMSC	Rabbit Model	n=25	Intramyocardial injection injecting into the border area of the ischemia myocardium.	4 weeks	1) ✅ Significant improvement in cardiac function 2) ✅ Significant infarct size reduction
Gandia *et al.*(24) 2008	Experimental animal study	Human DPSCs	Rat Model	n=16	Intramyocardial transplantation in five injections at five points of the infarct border	4 weeks	1) ✅ Significant improvement in cardiac function 2) ✅ Significant infarct size reduction 3) ✅ Significant vascular density increase
Liu et al.(21) 2008	Experimental animal study	Human BMMSC	Rat Model	n=23	Intramyocardial injection at the left anterior free wall	4 weeks	1) ✅ Significant improvement in cardiac function 2) ✅ Significant infarct size reduction 3) ✅ Significant vascular density increase
Shyu et al.(22) 2006	Experimental animal study	Human BMMSC	Mouse model	n=14	Intramyocardial injection	4 weeks	1) ✅ Significant improvement in cardiac function 2) ✅ Significant infarct size reduction 3) ✅ Significant vascular density increase
Rasmussen *et al.*(23) 2014	Experimental animal study	80 years old Human BMMSC	Rat Model	n=16	Intramyocardial injection	4 weeks	1) ❌ Non-significant results in cardiac function 2) ❌ Non-significant results in infarct size reduction 3) ❌ Non-significant results in vascular density increase

Table 2. Echocardiographic parameters of LVDd, LVDs and FS by study

DPSCs	**LVDd (mm) Control 4 weeks**	**LVDd (mm) DPSCs 4 weeks**	**LVDs (mm) Control 4 weeks**	**LVDs (mm) DPSCs 4 weeks**	**FS% Control 4 weeks**	**FS% DPSCs 4 weeks**	**FS evolution %**
Gandia *et al.* (24)	7.60 ± 0.18	7.26 ± 0.32	5.63 ± 0.23	4.94 ± 0.24	24.2 ± 1.6	32.9 ± 1.6	+ 36.95 %
Weighted mean DPSCs: + 35.95% FS							
hBMMSC	**LVDd (mm) Control 4 weeks**	**LVDd (mm) hBMMSC 4 weeks**	**LVDs (mm) Control 4 weeks**	**LVDs (mm) hBMMSC 4 weeks**	**FS% Control 4 weeks**	**FS% hBMMSC 4 weeks**	**FS evolution %**
Wang *et al.* (20)	15.4 ± 1.4	11.2 ± 1.8	9.7 ± 0.5	6.7 ± 0.5	37.7 ± 3.28	40.2 ± 3,3	+ 6.68%
Liu *et al.* (21)	7.8 ± 0.2	6.5 ± 0.1	5.4 ± 0.6	3.0 ± 0.1	31.5 ± 6.5	53.5 ± 1.7	+ 69.84%
Shyu *et al.* (22)	4.6 ± 0.2	3.6 ± 0.1	3.1 ± 0.3	2.2 ± 0.1	34.2 ± 2.3	44.4 ± 2.3	+ 30.01%
Rasmussen *et al.* (23)	-	-	-	-	-	-	Non-significant= 0%
Weighted mean hBMMSC: +27.13% FS							
SHED	**LVDd (mm) Control 4 weeks**	**LVDd (mm) SHED 4 weeks**	**LVDs (mm) Control 4 weeks**	**LVDs (mm) SHED 4 weeks**	**FS% Control 4 weeks**	**FS% SHED 4 weeks**	**FS evolution %**
Petchdee *et al.* (19)	-	-	-	-	-	-	Non-significant= 0%

Table 3. Infarct size reduction results

	Infarct size Control group after 4 weeks (mm)	Infarct size Treated group after 4 weeks (mm)	Infarct size evolution %
DPSCs			
Gandia *et al.* (24)	21.2 ± 1.6 %	15.9 ± 1.7 %	-25%
Weighted mean reduction of infarct size: - 25%			
SHED			
Petchdee *et al.* (19)	19.9 ± 0,03 %	10.9 ± 0.02 %	-45.2%
Weighted mean reduction of infarct size: - 45.2%			
hBMMSC			
Liu *et al.* (21)	54.9±3.3%	35.4±3.4%	-35.5%
Rasmussen *et al.* (23)	-	-	Non-significant = 0%
Shyu *et al.* (22)	66.1±1.5%	58.1±1.1%	-12.1%
Weighted mean reduction of infarct size: -18.67%			

Table 4. Descriptive results of vascular density evolution

	Control group vascular density after 4 weeks number of vessels per unit area (mm^2)	Treated group vascular density after 4 weeks number of vessels per unit area (mm^2)	Vascular density evolution %
DPSCs			
Gandia *et al.* (24)	518 ± 115/mm^2	868 ± 85/mm^2	+ 67.51%
Weighted mean increase of vascular density: + 67.51%			
SHED			
Petchdee *et al.* (19)	-	-	Increased number of capillaries based on H&E staining but not quantified.
Weighted mean increase of vascular density: Not quantified			
hBMMSC			
Liu *et al.* (21)	144 ± 16/mm^2	938±42/mm^2	+84.64%
Rasmussen *et al.* (23)	-	-	Non-significant = 0%
Shyu *et al.* (22)	439±27/mm^2	1119±17/mm^2	+60.8%
Weighted mean increase of vascular density: + 34.57%			

POTENCIAL TERAPÉUTICO DE LAS CÉLULAS MADRE DE LA PULPA DENTAL HUMANA EN LA REPARACIÓN CARDIACA, ¿UNA NUEVA ESPERANZA EN LA MEDICINA REGENERATIVA INTERDISCIPLINAR? - UNA REVISIÓN SISTEMÁTICA

Título corto: Potencial terapéutico de las células madre de la pulpa dental humana en la reparación cardiaca, ¿una nueva esperanza en la medicina regenerativa interdisciplinar?

Autores:

Emilie Caulet [1], María del Carmen Carceller Zazo [1]

[1] Universidad Europea de Valencia. Facultad de Ciencias de la Salud. Departamento de Odontología,

Correspondencia y reimpresión:

María del Carmen Carceller Zazo
Paseo Alameda 7,Valencia
46010, Valencia

carmen.carceller@universidadeuropea.es

Resumen:

Introducción: En las 2 últimas décadas, las células madre mesenquimales (MSCs) se han convertido en la base del campo emergente de la medicina regenerativa debido a su capacidad de autorrenovación y diferenciación multilinaje. A pesar de los avances en el tratamiento, el infarto de miocardio sigue siendo una causa importante de mortalidad y discapacidad. Las células madre de la pulpa dental (DPSCs) ofrecen prometedoras propiedades regenerativas para la regeneración cardiaca.

Objetivos: El objetivo de esta revisión es evaluar los efectos de las DPSCs humanas y las células madre de dientes temporales exfoliados humanos (SHED) en la función cardiaca, la reducción del tamaño del infarto y la angiogénesis en comparación con las MSCs de médula ósea humana (hBMMSC) en modelos de infarto de miocardio en roedores.

Material y métodos: Se ha realizado una búsqueda electrónica en las bases de datos PubMed, Scopus y Web of Science sobre regeneración cardiaca lograda por DPSCs, SHED o hBMMSC en modelos de infarto de miocardio en roedores. La búsqueda se completó mediante búsqueda manual. La fecha límite fue enero de 2023.

Resultados: De los 844 estudios identificados a través del proceso de búsqueda, se han incluido 6 estudios por cumplir los criterios de inclusión: 1 estudio describe el trasplante de SHED, 4 describen el trasplante de hBMMSC y 1 describe el trasplante de DPSCs. La regeneración cardiaca se ha evaluado a través de 3 parámetros: mejora de la función cardiaca (a través de la evolución de la fracción de acortamiento (FS)), reducción del tamaño del infarto y angiogénesis. En cuanto la FS, las DPSCs mostraron un aumento medio del 36,95%, las hBMMSC del 27,13%, y el grupo con SHED no proporcionó ningún resultado significativo. El tamaño del infarto se redujo en un 25% con DPSCs, un 45,2% con SHED y un 18,67% con hBMMSC. La angiogénesis se observó en todos los grupos pero no se cuantificó en el grupo SHED, el grupo DPSCs presentó un aumento medio de la densidad vascular del 67,51% y el grupo hBMMSC del 34,57%.

Conclusiones: A pesar de las limitaciones, las células madre de la pulpa dental parecen lograr una mayor mejora de la función cardiaca, la reducción del tamaño del infarto, así como la inducción de la angiogénesis en comparación con hBMMSC en modelos de roedores de infarto de miocardio.

Palabras clave: *Células madre de la pulpa dental humana, Células madre de la pulpa dental aisladas de la pulpa de dientes caducos exfoliados humanos, Células madre mesenquimales derivadas de médula ósea humana, Reparación cardiaca.*

INTRODUCCIÓN:

En un mundo en constante evolución, la odontología ha dejado constancia recientemente de su presencia a través de importantes saltos en la investigación médica interdisciplinar. Los profesionales de la odontología buscan constantemente mejorar el tratamiento de los pacientes. En las dos últimas décadas, las células madre mesenquimales (MSCs) se han convertido en la base del emergente campo de la medicina regenerativa gracias a sus capacidades de autorrenovación y diferenciación multilinaje (1). Aunque la capacidad regenerativa del tejido dental humano es limitada, el descubrimiento de células madre de la pulpa dental (DPSCs) de dentición permanente, así como de células madre de la pulpa dental aisladas de la pulpa de dientes deciduos exfoliados humanos (SHED) a principios de la década de 2000, ha abierto nuevos y sorprendentes horizontes en la regeneración dental, así como en la regeneración de tejidos no dentales, abriendo el camino a la colaboración interdisciplinar (2,3). A pesar de los recientes avances sustanciales en la prevención y el tratamiento, el infarto de miocardio y las enfermedades cardiovasculares siguen siendo una de las principales causas de muerte, con una prevalencia de 3 millones de personas al año en todo el mundo, lo que provoca daños irreversibles en los cardiomiocitos debido a la interrupción del suministro de oxígeno (4). La falta de capacidad regenerativa del miocardio pone de manifiesto la importancia de reducir la pérdida de cardiomiocitos, estimular la neovascularización y mantener la función cardiaca. Así pues, el trasplante de células madre dentales parece ser una opción sólida en el campo de la medicina regenerativa y actualmente ofrece resultados prometedores en la regeneración cardiaca. Aunque la mayoría de los estudios actuales se han realizado con células mesenquimales derivadas de médula ósea humana (hBMMSC), las DPSCs y las SHED mostraron una tasa de proliferación mayor que las hBMMSC, así como un potencial de diferenciación en miocitos, melanocitos, neuronas activas, células de tipo hepático, células productoras de insulina y cardiomiocitos. (5-11). La terapia con células madre mesenquimales y la regeneración cardiaca se basan principalmente en mecanismos indirectos (señalización paracrina) y directos (transdiferenciación), así como en la neovascularización, la inmunomodulación y la remodelación cardiaca. (12-14).

En esta revisión sistemática pretendemos comparar el potencial terapéutico de las células madre de la pulpa dental humana (DPSCs y SHED) frente a las hBMMSC en la reparación miocárdica tras un infarto de miocardio en roedores. En primer lugar, se ha evaluado la función cardiaca mediante la comparación de la evolución de la fracción de acortamiento con

el trasplante de DPSCs o SHED frente al trasplante de hBMMSC. En segundo lugar, se ha comparado la reducción del tamaño del infarto y la angiogénesis entre los grupos.

MATERIAL Y MÉTODOS:

La presente revisión sistemática se realizó siguiendo la declaración de directrices PRISMA (2020) (Preferred Reporting Items for Systematic reviews and Meta- Analyses) (15).

-Pregunta de enfoque: La pregunta de enfoque se estableció de acuerdo con la pregunta estructurada PICO. El formato de la pregunta fue el siguiente:

- **P** (Población): Modelos de infarto de miocardio en roedores
- **I** (Intervención): Trasplante SHED o DPSCs
- **C** (Comparación): trasplante de hBMMSC
- **O** (Resultados):

O1: Mejora de le función cardiaca

O2: Reducción del tamaño del infarto

O3: Inducción de la angiogénesis

-Criterios de inclusión:

Los criterios de inclusión fueron:

• Diseño de los estudios: Estudios con animales de experimentación aprobados éticamente; estudios publicados en inglés, francés o español publicados hasta enero de 2023.

-Pregunta del estudio: La pregunta del estudio se estableció de acuerdo con la pregunta estructurada PICO. El formato de la pregunta fue el siguiente:

- Población: Modelos *in vivo* de infarto de miocardio en roedores. Especies: Ratas, Ratones, Conejos. Sexo: roedor macho o hembra.
- Intervención: Trasplante de DPSCs o SHED o hBMMSC.
- Resultados: Estudios que miden la mejora de la función cardiaca como resultado primario, la reducción del tamaño del infarto y la inducción de angiogénesis como resultados secundarios.

Los criterios de exclusión fueron revisiones por pares, revisiones, cartas o comentarios al editor opiniones de expertos y editoriales, ensayos clínicos aleatorizados, series de casos, estudios en humanos. Además, se excluyeron todos los estudios realizados con células madre animales. No se aplicó ninguna restricción sobre la fecha de publicación.

- Fuentes de información y búsqueda de datos:

Se realizó una búsqueda en las tres bases de datos siguientes: PubMed, Scopus y Web of Science utilizando las siguientes palabras clave: “myocardial infarction”, “rodentia”, “acute myocardial infarction”, “chronic myocardial infarction”, “rodents”, “myocardial ischemia”, “reperfusion”, “human dental pulp stem cells”, “DPSC”, “dental stem cell therapy”, “stem cells from human exfoliated deciduous teeth”, “human Bone marrow-derived mesenchymal stem cells”, “BM-MSC”, “BMSC”, “human bone marrow multipotent mesenchymal stem cells”, “hMSCs", “cardiomyocytic differentiation” “cardiac repair”, “ventricular function”, “myocardial performance”, “cardiac function”. Las palabras clave se combinaron con los operadores booleanos AND y OR, así como con términos controlados ("MESH" para PubMed) con el fin de obtener los mejores y más amplios resultados de búsqueda.

Se llevó a cabo la siguiente estrategia de búsqueda en Pubmed: (((((((("Myocardial infarction" [MeSH Terms]) AND ("Rodentia"[MeSH Terms])) OR (Acute myocardial infarction)) OR (Chronic myocardial infarction)) AND (rodents) OR (myocardial ischemia))) AND ((((("Human dental pulp stem cells") OR ("DPSC")) OR (dental stem cell therapy)) OR ("Stem cells from human exfoliated deciduous teeth"))) OR (((((("Human Bone marrow-derived mesenchymal stem cells") OR ("BM-MSC")) OR ("BMSC")) OR (human bone marrow multipotent mesenchymal stem cells)) OR ("hMSCs"))) AND (((((("Cardiomyocytic differentiation") OR (cardiac repair)) OR ("ventricular function")) OR ("myocardial performance") OR ("cardiac function")) OR ("reperfusion")).

Para el análisis se llevó a cabo una búsqueda cruzada de publicaciones posiblemente interesantes. Se contactó con los autores de los trabajos para adquirir aquellos que no estaban en bases de datos de texto completo. Se eliminaron de la revisión los estudios duplicados. La búsqueda finalizó con una comprobación de las referencias que figuraban en la bibliografía de cada estudio para encontrar cualquier investigación adecuada que la búsqueda inicial pudiera

haber pasado por alto. Se realizaron búsquedas manuales de literatura científica en las publicaciones *Journal of the American College of Cardiology* y *Medwell Journals.*

- Estrategia de búsqueda:

Dos revisores (EC, CC) llevaron a cabo un proceso de selección en tres fases. En la fase preliminar se analizaron los títulos y se descartaron las publicaciones irrelevantes. La segunda fase se centró en los resúmenes, se realizó un cribado y la selección se basó en el tipo de estudio, el tipo de trasplante de células madre, el tipo de modelo de infarto de miocardio y las variables de resultado. En la última fase se examinó el texto completo y se extrajeron los datos mediante un formulario de recogida de datos preparado previamente para confirmar la elegibilidad de los estudios. Los desacuerdos entre los revisores, en cada una de las fases, se resolvieron mediante discusión y, cuando fue necesario, se consultó a un tercer revisor. El grado de acuerdo con respecto a la inclusión de estudios potenciales se calculó mediante la prueba kappa de Cohen (estadística k) para la segunda y tercera etapas de selección.

- Extracción de datos:

Se extrajo información de los estudios y se organizó en tablas según los autores con el año de publicación, el tipo de estudio, el tipo de trasplante de células madre (DPSCs, SHED o hBMMSC), el tipo de modelo de lesión miocárdica (ratas, ratones, conejos), el tamaño de la muestra, el tipo de vía de administración (intravenosa o intramiocárdica), el tiempo transcurrido tras el trasplante (semanas). Se analizaron parámetros ecocardiográficos como: la dimensión interna del ventrículo izquierdo al final de la diástole (LVDd) y al final de la sístole (LVDs) en milímetros, así como el acortamiento fraccional (FS) en porcentajes, 4 semanas después del trasplante y se compararon con los grupos control. El acortamiento no fraccional se calculó según la siguiente fórmula FS= [[(LVDd - LVDs)/LVDd]*100]. (16). Se tiñeron los cortes transversales de los corazones extirpados, la extensión del tamaño del infarto (área del infarto/área ventricular izquierda total) se expresó como porcentaje del área ventricular izquierda total como media de todos los cortes de cada corazón. Los resultados se analizaron en porcentajes y se compararon con los del grupo de control. La densidad vascular se evaluó mediante inmunotinción. A continuación, el número de vasos por unidad de superficie (mm^2) se expresó en porcentajes de cambio y se comparó con los grupos de control.

- Evaluación de la calidad y del riesgo de sesgo:

La evaluación de la calidad de los estudios experimentales con animales se basó en las directrices ARRIVE 2.0 (Animal Research: Reporting *in vivo* Experiment) y fue realizada por dos revisores (EC,CC) (17). Se examinaron los artículos y se evaluaron los sesgos según la valoración de los 21 ítems (10 de los "10 esenciales" y 11 del "conjunto recomendado"): si una publicación satisfacía todos los subítems, se puntuaba como "informada" y se le otorgaban 2 puntos. Si una publicación no satisfacía ninguno de los subapartados, se calificaba como "no comunicada" y se le otorgaban 0 puntos. Si los detalles proporcionados para los subapartados no estaban claros, se puntuó como "poco claro" y se le dio 1 punto. De este modo se determinó la calidad del estudio aplicando un coeficiente preestablecido, que oscilaba entre 0,8 y 1 para una calidad excelente, entre 0,5 y 0,8 para deficiente. El coeficiente se calculó sumando el total de puntos obtenidos por cada estudio y dividiéndolo por el máximo posible de puntos por estudio, que era 42. También se ha evaluado la calidad por dominios. El grado de acuerdo interexaminadores de la evaluación de la calidad metodológica se obtuvo con la prueba kappa de Cohen, siguiendo la escala propuesta por Landis y Koch (18).

- Síntesis de datos:

Se realizó un análisis descriptivo de las variables de resultado. Dado que las medias calculadas en los estudios analizados procedían de muestras de roedores de diferente tamaño, fue necesario calcular la media ponderada con el objetivo de obtener resultados más precisos. Las medias de los valores de las variables primarias se agruparon por grupos de investigación (DPSCs, SHED y hBMMSC). Debido a la falta de estudios aleatorizados que compararan todos los grupos de tratamiento, no fue posible realizar un metaanálisis, por lo que los resultados se concentraron en un análisis descriptivo de los factores.

RESULTADOS:

- Selección de estudios:

Se identificaron un total de 843 estudios mediante el proceso de búsqueda inicial: Medline-PubMed (n=366), SCOPUS (n=48) y Web of Science (n=429). Se obtuvo un estudio adicional mediante búsqueda manual (lista de referencias y fuentes primarias). De estas publicaciones, 24 se identificaron como potencialmente elegibles mediante el cribado de los títulos. El cribado de los resúmenes de estas 24 publicaciones condujo a la selección de 15 artículos. Por último, se exanimaron los artículos a texto completo, lo que condujo a la selección final de los 6 artículos incluidos en la presente revisión sistemática (Fig. 1). El valor k para el acuerdo entre examinadores sobre la inclusión de estudios fue de 0,87 (títulos y resúmenes) y 1,0 (textos completos), lo que indica un acuerdo "bueno" y "completo", respectivamente, según los criterios de Landis y Koch (18).

- Características del estudio:

De los 6 artículos seleccionados en la presente revisión, uno describe el trasplante de SHED (19). Cuatro estudios describen el trasplante de hBMMSC (20-23) y uno describe el trasplante de DPSCs (24). Todos ellos son estudios experimentales con animales. Un total de 110 roedores fueron inducidos con infarto de miocardio, de los cuales 54 roedores han sido tratados con células madre mesenquimales humanas incluyendo 8 roedores tratados con SHED, 7 roedores tratados con DPSC y 39 fueron tratados con hBMMSC. En 2 estudios se utilizó un modelo de infarto de miocardio en conejo (19,20), 3 utilizaron un modelo de rata (21,23,24) y 1 utilizó un modelo de ratón (22). En cuanto a la vía de administración de las células madre mesenquimales, 5 estudios utilizaron la inyección intramiocárdica (20- 24) y 1 utilizó una inyección intravenosa (19). Todos los datos se recogieron 4 semanas después del trasplante (Tabla 1).

- Riesgo de sesgo:

En esta revisión, dos estudios fueron calificados como de excelente calidad (22,24) mientras que los cuatro estudios restantes se clasificaron como de calidad media (19-21,23). El ítem que planteó el mayor riesgo de sesgo fue el de alojamiento y cría, como se indica en la Figura 3. El valor k (prueba kappa de Cohen) para el acuerdo entre revisores sobre la calidad metodológica fue de 0,9 según la escala de Landis & Koch (18).

-Síntesis de los resultados:

Mejora de la función cardiaca:

Los estudios realizados por Petchdee *et al*. (19) con SHED y Rasmussen *et al.* (23) con hBMMSC no aportaron resultados significativos sobre la evolución de la FS entre el grupo de control y los grupos tratados. Gandia *et al*. (24) observaron un aumento de la FS del 36,95% en comparación con el grupo control 4 semanas después de la inyección intramiocárdica de DPSCs en el borde del infarto. El grupo control presentó un deterioro significativo de la función cardiaca y de las dimensiones ventriculares internas. El aumento medio ponderado de la FS en el grupo de hBMMSC fue del 27,13% en comparación con el grupo control 4 semanas después de las inyecciones intramiocárdicas (20-23). El mayor aumento de la FS lo obtuvieron Liu *et al*. (21) con un 69,84%. El menor lo obtuvieron Wang *et al.* (20) con un aumento del 6,68%. Los grupos control presentaron una disminución significativa de la función cardiaca y de las dimensiones ventriculares. El único estudio que no obtuvo resultados de mejoría de la función cardiaca con hBMMSC fue el realizado por Rasmussen et al. (23). Por otro lado, el grupo SHED no mostró una mejora significativa de la función cardiaca (19). La mayor mejora de la función cardiaca se obtuvo con DPSCs (24) con una diferencia significativa ($p=0,010$) en comparación con las hBMMSC. Los resultados descriptivos de los parámetros ecocardiográficos se muestran en la Tabla 2.

Reducción del tamaño del infarto:

Los tres tipos de tratamiento con células madre: DPSCs, SHED y hBMMSC mostraron resultados significativos ($p<0,5$) en la reducción del tamaño del infarto. En el estudio de Gandia *et al.* (24) las DPSCs mostraron una reducción del tamaño del infarto del 25% en comparación con el grupo control 4 semanas después de la inyección intramiocárdica en el borde del infarto. Petchdee *et al*. (19) observaron una reducción del 45,2% del área del infarto con la inyección intravenosa de SHED. En cuanto al grupo de hBMMSC, Rasmussen et al. (23) realizaron el único estudio dentro de este grupo que no consiguió una reducción significativa del área del infarto. La reducción media ponderada con hBMMSC fue del 18,67%. La mayor reducción del área del infarto la consiguió el grupo SHED con una reducción del 45,2%, seguido del grupo DPSCs con una reducción del 25% y la menor reducción la consiguió el grupo

hBMMSC con un 18,67%. Los resultados descriptivos de la reducción del tamaño del infarto se muestran en la Tabla 3.

Inducción de la angiogénesis:

Las DPSCs indujeron la angiogénesis, dando lugar a la formación de vasos sanguíneos funcionales derivados de ratas. Se produjo un aumento del 67,51% en la densidad vascular en comparación con el grupo de control (24). Petchdee *et al.* (19) observaron un aumento de la densidad vascular mediante secciones histológicas teñidas con hematoxilina y eosina (H&E). No fue posible realizar una comparación cuantitativa, ya que no se facilitaron los valores exactos. El aumento medio ponderado para el grupo de hBMMSC fue del 34,57%, siendo el aumento más alto del 84,64% el obtenido por Liu *et al*. (21). Una vez más, el estudio realizado por Rasmussen *et al*. (23) no obtuvo resultados significativos en la inducción de la angiogénesis. Los resultados descriptivos de la evolución de la densidad vascular se presentan en la Tabla 4.

DISCUSIÓN:

Mejora de la función cardiaca:

Los resultados de esta revisión sistemática basada en seis investigaciones científicas revelaron una mayor mejora de la función cardiaca con la inyección intramiocárdica de DPSCs en comparación con las hBMMSC y las SHED. La falta de resultados en la regeneración cardiaca observada por Rasmussen *et al.* (23) tras el trasplante de hBMMSC puede atribuirse al hecho de que las células madre utilizadas en el experimento procedían de un donante de 80 años de edad. De hecho, Fan *et al. (*25) et al y Liu *et al*. (26) han analizado la importancia de la edad avanzada en las capacidades cardioprotectoras y regenerativas de las células madre mesenquimales. Ambos estudios demostraron que la edad tenía un impacto sustancial en la capacidad de regeneración de las hBMMSC. Los trasplantes de hBMMSC jóvenes mejoraron los resultados funcionales tras un IM al detener la degradación de la matriz y fomentar la angiogénesis (25,26). En 2017, Song *et al*. (27) realizaron un estudio en el que profundizaron en el rejuvenecimiento de hBMMSC y observaron su eficacia. Descubrieron que la mejora del rendimiento cardiaco tras un infarto de miocardio se obtenía en corazones de ratones mediante el trasplante de hBMMSC envejecidas que sobreexpresaban el factor neurotrófico

derivado de neuronas, lo que mejoraba la supervivencia de las células madre implantadas. En cuanto a las SHED, aunque no aportaron resultados significativos en la mejora de la función cardiaca, validaron la seguridad del trasplante de SHED tras un infarto de miocardio en un modelo de conejo (19). Un estudio realizado por Yamaguchi *et al*. (28) proporcionó la primera prueba de que el medio de cultivo condicionado SHED confería resistencia al daño isquémico agudo en el corazón: de hecho, el medio de cultivo condicionado SHED aumentó significativamente la FS ventricular, suprimió la apoptosis y la inflamación en el corazón isquémico de ratones 7 días después de la inducción del infarto de miocardio.

Reducción del tamaño del infarto:

La reducción puede explicarse mediante el examen ultraestructural de la pared ventricular izquierda y las zonas periinfarto, que mostró un aumento de los haces de cardiomiocitos que disminuyó el tamaño del infarto y favoreció una mayor fracción de miofibroblastos. Los miofibroblastos están relacionados con la recuperación de las heridas isquémicas, y su ontogénesis está relacionada con las alteraciones morfológicas observadas en los fibroblastos sometidos a tensión de estrés (19,24). Mientras que las DPSCs no se diferenciaron en cardiomiocitos, las hBMMSC injertadas presentaron una diferenciación cardiomiocítica que les confirió la capacidad de sobrevivir a largo plazo en el tejido cardiaco (20-22,24). Un estudio *in vitro* realizado por Kittivarakam *et al*. (29) demostró que las SHED podían diferenciarse en cardiomiocitos funcionales y eran capaces de proliferar en andamios electrospun. También podemos añadir que a través de periodos de tiempo más largos las células madre mesenquimales de médula ósea alogénicas necesitaron entre 3 y 6 meses para expresar marcadores musculares, por lo que no se descarta la posibilidad de que sean necesarios periodos de trasplante más largos para inducir la expresión de marcadores cardíacos en las DPSCs trasplantadas (30). La edad del donante mermó la capacidad de las hBMMSC para reducir el tamaño del infarto. Rasmussen *et al*. (23) no observaron ningún resultado utilizando las hBMMSC del donante de 80 años.

Inducción de la angiogénesis:

En esta revisión observamos que las DPSCs conseguían una mayor inducción de angiogénesis con un mayor porcentaje de aumento de la densidad vascular en comparación con las hBMMSC. La angiogénesis desencadenada por SHED no se ha comparado porque el estudio

no proporcionó datos cuantitativos. Shyu *et al*. (22) demostraron que los hBMMSC presentan un potencial superior para potenciar la angiogénesis que el factor de crecimiento endotelial vascular (VEGF) y los factores de crecimiento angiogénicos tras un infarto de miocardio en ratones. La edad del donante de hBMMSC también desempeñó un papel en la inducción de la angiogénesis, ya que Rasmussen *et al*. (23) observaron que las hBMMSC de 80 años de edad no proporcionaban ningún resultado significativo en la inducción de la angiogénesis. Fan et *al*. (25). Por otra parte, observaron que los niveles VEGF y proteína se elevaban significativamente en el miocardio tras el trasplante de hBMMSC jóvenes.

Limitaciones:

La presente revisión reveló una falta de estudios experimentales en animales centrados en las células madre de la pulpa dental, ya que en la actualidad sólo hay un estudio centrado en las DPSCs y el infarto de miocardio (24) y sólo hay un estudio similar centrado en SHED (19). Por este motivo, los resultados de la presente revisión sistemática deben interpretarse con cautela. Otra limitación es la falta de uniformidad en los datos recogidos acerca de la medición de la función cardiaca: los estudios sobre DPSCs carecían de datos sobre las cifras de fracción de eyección, y la fracción de acortamiento no siempre se midió directamente. A pesar de ello, los datos sobre la LVDd y la LVDs nos permitieron calcular la FS y comparar así la mejora conseguida por cada grupo de células (19,24). Un análisis más completo de la función cardiaca habría requerido mediciones de la fracción de eyección. Del mismo modo, existe una falta de uniformidad en la forma de medir la densidad capilar. Mientras que en algunos estudios se obtuvo mediante inmunotinción, en otros se evaluó a partir de fotomicrografías mediante análisis de imagen informatizado. La edad de las células donantes también influyó en los resultados y redujo drásticamente los resultados medios ponderados del grupo de hBMMSC. La falta de información sobre la edad de los donantes no nos permitió diferenciar los resultados dentro del grupo hBMMSC (23,25-27). Otra limitación fue la falta de tiempo de seguimiento para poder detectar posibles efectos secundarios oncogénicos o la diferenciación cardiomiocítica de las DPSCs.

Implicaciones clínicas y perspectivas de futuro

Las células madre de la pulpa dental representan una alternativa sustancial para la regeneración no dental y el tratamiento de diversas enfermedades inflamatorias gracias a sus propiedades inmunomoduladoras y su potencial de diferenciación. Las implicaciones preclínicas en estudios experimentales con animales han proporcionado resultados prometedores que abren el camino a nuevas investigaciones que fomenten la realización de ensayos clínicos para validar la seguridad y eficacia del trasplante de células madre de la pulpa dental. En el futuro, las células madre dentales también podrán combinarse con medio de cultivo condicionado, otros tipos de células madre, y factores de crecimiento que puedan mejorar sus propiedades en la regeneración miocárdica. Las mejoras actuales en los bancos de células madre de la pulpa dental, así como su fácil accesibilidad, abundancia, baja tumorigenicidad y aceptación ética, sitúan al humilde diente como un serio candidato para la medicina interregenerativa.

REFERENCIAS:

1. Aly LAA. Stem cells: Sources, and regenerative therapies in dental research and practice. World J Stem Cells. 2015;7(7):1047–53.
2. Yamada Y, Nakamura-Yamada S, Kusano K, Baba S. Clinical potential and current progress of dental pulp stem cells for various systemic diseases in regenerative medicine: A concise review. Int J Mol Sci .; 2019; 20(5):1132.
3. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA. 2000; 97(25):13625-30.
4. Kim SJ. Global awareness of myocardial infarction symptoms in general population. Vol. 51, Korean Circ J .2021; 51(12):997-1000.
5. Patil R, Kumar BM, Lee WJ, Jeon RH, Jang SJ, Lee YM, et al. Multilineage potential and proteomic profiling of human dental stem cells derived from a single donor. Exp Cell Res. 2014 ;320(1):92–107.
6. Ishkitiev N, Yaegaki K, Calenic B, Nakahara T, Ishikawa H, Mitiev V, et al. Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. J Endod. 2010;36(3):469–74.
7. Armiñán A, Gandía C, Bartual M, García-Verdugo JM, Lledó E, Mirabet V, et al. Cardiac differentiation is driven by NKX2.5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. Stem Cells Dev. 2009 ;18(6):907–17.
8. Heng BC, Lim LW, Wu W, Zhang C. An Overview of Protocols for the Neural Induction of Dental and Oral Stem Cells In Vitro. Tissue Eng Part B Rev. 2016 ;22(3):220–50.
9. Saghiri MA, Asatourian A, Sorenson CM, Sheibani N. Role of angiogenesis in endodontics: contributions of stem cells and proangiogenic and antiangiogenic factors to dental pulp regeneration. J Endod. 2015 ;41(6):797–803.

10. Stevens A, Zuliani T, Olejnik C, LeRoy H, Obriot H, Kerr-Conte J, et al. Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev. 2008;17(6):1175–84.
11. Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endod. 2009 ;35(11):1536–42.
12. Redgrave RE, Tual-Chalot S, Davison BJ, Singh E, Hall D, Amirrasouli MM, et al. Cardiosphere-Derived Cells Require Endoglin for Paracrine-Mediated Angiogenesis. Stem Cell Reports. 2017 ;8(5):1287.
13. Chimenti I, Smith RR, Li TS, Gerstenblith G, Messina E, Giacomello A, et al. Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice. Circ Res. 2010;106(5):971–80.
14. Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. Cell Mol Life Sci. 2017 ;74(13):2345–60.
15. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
16. Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ, et al.. New Index of Combined Systolic and Diastolic Myocardial Performance: A Simple and Reproducible Measure of Cardiac Function-A Study in Normals and Dilated Cardiomyopathy. J Cardiol. 1995;26(6):357–66.
17. Percie Du Sertid N, Hurst V, Ahluwaliaid A, Alamid S, Aveyid MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Br J Pharmacol. 2020; 177(16):3617-24.
18. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics. 1977 ;33(2):363–74.
19. Petchdee S, Pattanapon N, Bootcha R, Srivattanakul P, Songserm T. Dental tissue-derived stem cells exerts therapeutic effects on chronic myocardial infarction model of rabbit. The Cardiology. 2014;9:1–6.
20. Wang JA, Fan YQ, Li CL, He H, Sun Y, Lu BJ. Human bone marrow-derived mesenchymal stem cells transplanted into damaged rabbit heart to improve heart function. J Zhejiang Univ Sci B. 2005 ;6(4):242–8.
21. Liu JF, Wang BW, Hung HF, Chang H, Shyu KG. Human mesenchymal stem cells improve myocardial performance in a splenectomized rat model of chronic myocardial infarction. J Formos Med Assoc. 2008;107(2):165–74.
22. Shyu KG, Wang BW, Hung HF, Chang CC, Shih DTB. Mesenchymal stem cells are superior to angiogenic growth factor genes for improving myocardial performance in the mouse model of acute myocardial infarction. J Biomed Sci. 2006 ;13(1):47–58.
23. Rasmussen JG, Frøbert O, Holst-Hansen C, Kastrup J, Baandrup U, Zachar V, et al. Comparison of human adipose-derived stem cells and bone marrow-derived stem cells in a myocardial infarction model. Cell Transplant. 2014 ;23(2):195–206.
24. Gandia C, Armiñan A, García-Verdugo JM, Lledó E, Ruiz A, Miñana MD, et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells. 2008 ;26(3):638–45.
25. Fan M, Chen W, Liu W, Du GQ, Jiang SL, Tian WC, et al. The Effect of Age on the Efficacy of Human Mesenchymal Stem Cell Transplantation after a Myocardial Infarction. Rejuvenation Res. 2010; 13(4):429-38.

26. Liu Y, Liu T, Han J, Yang Z, Xue X, Jiang H, et al. Advanced age impairs cardioprotective function of mesenchymal stem cell transplantation from patients to myocardially infarcted rats. Cardiology . 2014;128(2):209–19.
27. Song HF, He S, Li SH, Yin WJ, Wu J, Guo J, et al. Aged Human Multipotent Mesenchymal Stromal Cells Can Be Rejuvenated by Neuron-Derived Neurotrophic Factor and Improve Heart Function After Injury. JACC Basic Transl Sci. 2017 ;2(6):702–16.
28. Yamaguchi S, Shibata R, Yamamoto N, Nishikawa M, Hibi H, Tanigawa T, et al. Dental pulp-derived stem cell conditioned medium reduces cardiac injury following ischemia-reperfusion. Sci Rep. 2015 ;5:16295.
29. Kittivarakarn P, Penna M, Acosta Z, Pelaez D, Montero R, Andreopoulos FM, et al. Cardiomyotic induction and proliferation of dental stem cells on electrospun scaffolds. AIMS Bioengineering. . 2016;3(2):139–55.
30. Dai W, Hale SL, Martin BJ, Kuang JQ, Dow JS, Wold LE, et al. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. Circulation. 2005 ;112(2):214–23.

Financiación: *Ninguna declarada.*

Conflicto de intereses: *Ninguno declarado.*

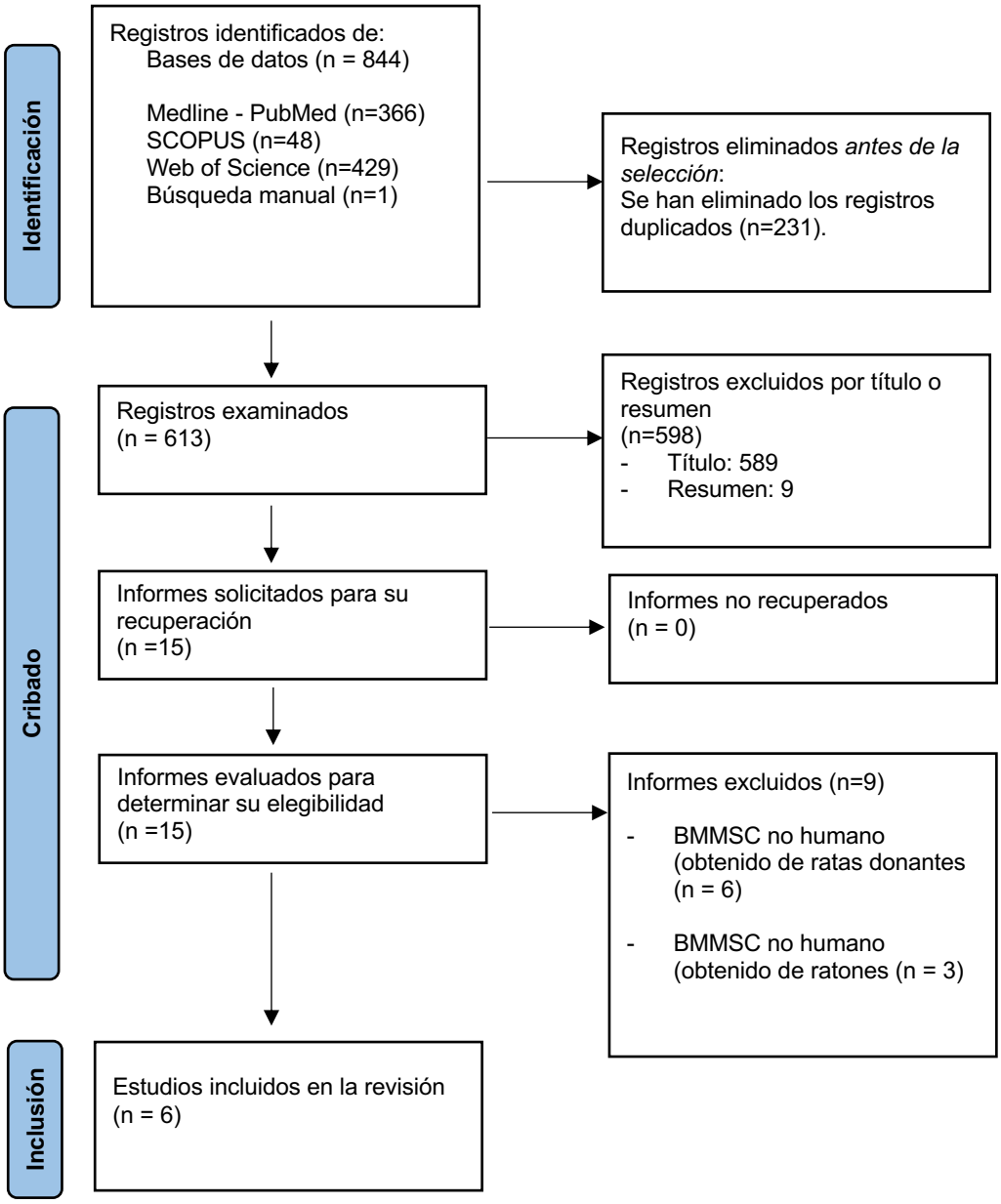

Figura.1. Diagrama de flujo de búsqueda y proceso de selección de títulos de nuestra revisión sistemática.

	Soontaree *et al.* Año 2014	Wang *et al.* Año 2005	Gandia *et al.* Año 2008	Liu *et al.* Año 2008	Shyu *et al.* Año 2006	Rasmussen *et al.* Año 2014
1. Diseño del estudio Para cada experimento, proporcione detalles breves del diseño del estudio, incluyendo: a. Los grupos que se comparan, incluidos los grupos de control. Si no se ha utilizado ningún grupo de control, se debe indicar la justificación. b. La unidad experimental (por ejemplo, un solo animal, camada o jaula de animales).	2	2	2	2	2	2
2. Tamaño de la muestra a. Especifique el número exacto de unidades experimentales asignadas a cada grupo y el número total en cada experimento. Indíquese también el número total de animales utilizados. b. Explique cómo se decidió el tamaño de la muestra. Proporcione detalles de cualquier *cálculo de tamaño de muestra a priori*, si se hace.	1	1	2	1	2	1
3. Criterios de inclusión y exclusión a. Describa cualquier criterio utilizado para incluir y excluir animales (o unidades experimentales) durante el experimento, y los puntos de datos durante el análisis. Especifique si estos criterios se establecieron *a priori.* Si no se establecieron criterios, indíquelo explícitamente. b. Para cada grupo experimental, informe cualquier animal, unidad experimental o punto de datos no incluido en el análisis y explique por qué. Si no hubo exclusiones, indíquelo. c. Para cada análisis, informe el valor exacto de *n* en cada grupo experimental.	2	2	2	2	2	2
4. Aleatorización a. Indique si se utilizó la aleatorización para asignar unidades experimentales a los grupos de control y tratamiento. Si lo hace, proporcione el método utilizado para generar la secuencia de aleatorización. b. Describa la estrategia utilizada para minimizar los posibles factores de confusión, como el orden de los tratamientos y mediciones, o la ubicación del animal / jaula. Si los factores de confusión no fueron controlados, indíquelo explícitamente.	1	1	1	1	1	1
5. Cegamiento Describa quién estaba al tanto de la asignación de grupos en las diferentes etapas del experimento (durante la asignación, la realización del experimento, la evaluación de resultados y el análisis de datos).	0	0	2	2	2	2
6. Medidas de resultado a. Definir claramente todas las medidas de resultado evaluadas (por ejemplo, muerte celular, marcadores moleculares o cambios de comportamiento). b. Para los estudios de prueba de hipótesis, especifique la medida de resultado primaria, es decir, la medida de resultado que se utilizó para determinar el tamaño de la muestra.	2	2	2	2	2	2
7. Métodos estadísticos a. Proporcione detalles de los métodos estadísticos utilizados para cada análisis, incluido el software utilizado. b. Describa cualquier método utilizado para evaluar si los datos cumplían con los supuestos del enfoque estadístico y qué se hizo si no se cumplieron los supuestos.	2	2	2	2	2	2

8. Animales de experimentación a. Facilítese información apropiada para cada especie de los animales utilizados, incluidas las especies, la cepa y la subestirpación, el sexo, la edad o la etapa de desarrollo y, si procede, el peso. b. Proporcionar más información relevante sobre la procedencia de los animales, el estado de salud / inmunológico, el estado de modificación genética, el genotipo y cualquier procedimiento anterior.	1	1	1	1	1	1
9. Procedimientos experimentales Para cada grupo experimental, incluidos los controles, describa los procedimientos con suficiente detalle para permitir que otros los repliquen, incluyendo: a. Qué se hizo, cómo se hizo y qué se utilizó. b. Cuándo y con qué frecuencia. c. Dónde (incluyendo detalle de cualquier período de aclimatación). d. Por qué (proporcionar justificación para los procedimientos).	1	1	1	1	1	1
10. Resultados Para cada experimento realizado, incluidas las réplicas independientes, informe: a. Resumen / estadística descriptiva para cada grupo experimental, con una medida de variabilidad cuando corresponda (por ejemplo, media y DE, o mediana y rango). b. Si procede, el tamaño del efecto con un intervalo de confianza.	2	2	2	2	2	2
11. Resumen Proporcione un resumen preciso de los objetivos de la investigación, las especies animales, la cepa y el sexo, los métodos clave, los hallazgos principales y las conclusiones del estudio.	2	2	1	1	1	2
12. Antecedentes a. Incluya suficientes antecedentes científicos para comprender la justificación y el contexto del estudio, y explique el enfoque experimental. b. Explique cómo la especie animal y el modelo utilizado abordan los objetivos científicos y, en su caso, la relevancia para la biología humana.	2	2	2	2	2	2
13. Objetivos Describa claramente la pregunta de investigación, los objetivos de la investigación y, en su caso, las hipótesis específicas que se están probando.	2	2	2	2	2	2
14. Declaración ética Proporcione el nombre del comité de revisión ética o equivalente que haya aprobado el uso de animales en este estudio, y cualquier número de licencia o protocolo relevante (si corresponde). Si no se solicitó o concedió la aprobación ética, proporcione una justificación.	2	2	2	2	2	2
15. Vivienda y cría Proporcione detalles de las condiciones de vivienda y cría, incluido cualquier enriquecimiento ambiental.	0	0	0	0	0	0
16. Cuidado y monitoreo de animales a. Describir cualquier intervención o paso tomado en los protocolos experimentales para reducir el dolor, el sufrimiento y la angustia. b. Informe cualquier evento adverso esperado o inesperado. c. Describa los criterios de valoración humanitarios establecidos para el estudio, los signos que fueron monitoreados y la frecuencia del monitoreo. Si el estudio no tuvo criterios de valoración humanitarios, indíquelo.	1	1	1	1	1	1
17. Interpretación/implicaciones científicas a. Interpretar los resultados, teniendo en cuenta los objetivos e hipótesis del estudio, la teoría actual y otros estudios relevantes en la literatura. b. Comente sobre las limitaciones del estudio, incluidas las posibles fuentes de sesgo, las limitaciones del modelo animal y la imprecisión asociada con los resultados.	2	2	2	2	2	2

18. Generalizabilidad/traducción Comente si, y cómo, es probable que los hallazgos de este estudio se generalicen a otras especies o condiciones experimentales, incluida cualquier relevancia para la biología humana (cuando corresponda).	2	2	2	2	2	2
19.Registro del protocolo Proporcione una declaración que indique si se preparó un protocolo (incluida la pregunta de investigación, las características clave del diseño y el plan de análisis) antes del estudio, y si se registró este protocolo y dónde.	1	1	1	1	1	1
20. Acceso a los datos Proporcione una declaración que describa si los datos del estudio están disponibles y dónde.	2	2	2	2	2	2
21. Declaración de intereses a. Declarar cualquier posible conflicto de intereses, incluidos los financieros y no financieros. Si no existe ninguno, esto debe indicarse. b. Enumere todas las fuentes de financiamiento (incluido el identificador de la subvención) y el papel del financiador (s) en el diseño, análisis e informe del estudio.	2	0	2	2	2	2
Coeficiente:	32/42 **0,76**	30/42 **0,71**	34/42 **0,81**	33/42 **0,78**	34/42 **0,81**	34/42 **0,76**
Calidad:	**Promedio**	**Promedio**	**Excelente**	**Promedio**	**Excelente**	**Promedio**

Figura.2. Coeficientes de calidad de los estudios revisados según las directrices ARRIVE 2.0.

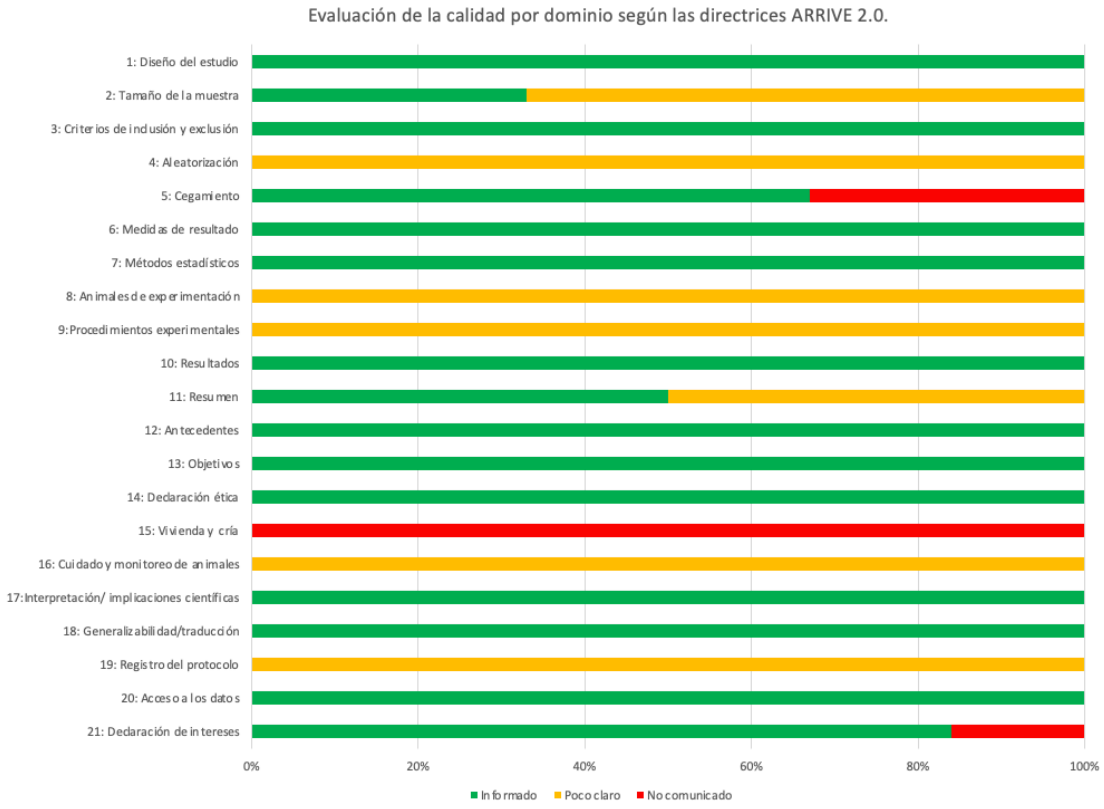

Figura.3. Evaluación de la calidad por dominio según las directrices ARRIVE 2.0.

Tabla 1. Características de los estudios revisados

Autores. Año	Tipo de estudio	Tipo de trasplantación	Modelo de infarto	Tamaño de la muestra	Vía de administración	Tiempo transcurrido tras de la trasplantación	Resultados
Petchdee *et al.*(19) 2014	Estudio experimental con animales	SHED	Modelo: conejo	n=16	Inyección intravenosa a través de la vena marginal del oído del conejo	4 semanas	1) ❌ Resultados no significativos en la función cardíaca 2) ✅ Reducción significativa del tamaño del infarto 3) ✅ Angiogénesis observada
Wang *et al.*(20) 2005	Estudio experimental con animales	hBMMSC	Modelo: conejo	n=25	Inyección intramiocárdica inyectando en el área fronteriza del miocardio isquemia.	4 semanas	1) ✅ Mejora significativa en la función cardíaca 2) ✅ Reducción significativa del tamaño del infarto
Gandia *et al.*(24) 2008	Estudio experimental con animales	DPSCs	Modelo: Rata	n=16	Trasplante intramiocárdico en cinco inyecciones en cinco puntos del borde del infarto	4 semanas	1) ✅ Mejora significativa en la función cardíaca 2) ✅ Reducción significativa del tamaño del infarto 3) ✅ Aumento significativo de la densidad vascular
Liu et al.(21) 2008	Estudio experimental con animales	BMMSC	Modelo: Rata	n=23	Inyección intramiocárdica en la pared libre anterior izquierda	4 semanas	1) ✅ Mejora significativa en la función cardíaca 2) ✅ Reducción significativa del tamaño del infarto 3) ✅ Aumento significativo de la densidad vascular
Shyu et al.(22) 2006	Estudio experimental con animales	BMMSC	Modelo: Ratón	n=14	Inyección intramiocárdica	4 semanas	1) ✅ Mejora significativa en la función cardíaca 2) ✅ Reducción significativa del tamaño del infarto 3) ✅ Aumento significativo de la densidad vascular
Rasmussen *et al.*(23) 2014	Estudio experimental con animales	BMMSC de un donante de 80 años	Modelo: Ratón	n=16	Inyección intramiocárdica	4 semanas	1) ❌ Resultados no significativos en la función cardíaca 2) ❌ Resultados no significativos en la reducción del tamaño del infarto 3) ❌ Resultados no significativos en el aumento de la densidad vascular

Tabla 2. Parámetros ecocardiográficos de LVDd, LVDs y FS por estudio

DPSCs	**LVDd (mm) Control 4 semanas**	**LVDd (mm) DPSCs 4 semanas**	**LVDs (mm) Control 4 semanas**	**LVDs (mm) DPSCs 4 semanas**	**FS% Control 4 semanas**	**FS% DPSCs 4 semanas**	**FS evolución %**
Gandia *et al.*(24)	7,60 ± 0,18	7,26 ± 0,32	5,63 ± 0,23	4,94 ± 0,24	24,2 ± 1,6	32,9 ± 1,6	+ 36,95 %
Media ponderada DPSCs: + 35,95% FS							
hBMMSC	**LVDd (mm) Control 4 semanas**	**LVDd (mm) hBMMSC 4 semanas**	**LVDs (mm) Control 4 semanas**	**LVDs (mm) hBMMSC semanas**	**FS% Control 4 semanas**	**FS% hBMMSC 4 semanas**	**FS evolución %**
Wang *et al.*(20)	15,4 ± 1,4	11,2 ± 1,8	9,7 ± 0,5	6,7 ± 0,5	37,7 ± 3,28	40,2 ± 3,3	+ 6,68%
Liu *et al.*(21)	7,8 ± 0,2	6,5 ± 0,1	5,4 ± 0,6	3,0 ± 0,1	31,5 ± 6,5	53,5 ± 1,7	+ 69,84%
Shyu *et al.*(22)	4,6 ± 0,2	3,6 ± 0,1	3,1 ± 0,3	2,2 ± 0,1	34,2 ± 2,3	44,4 ± 2,3	+ 30,01%
Rasmussen *et al.*(23)	-	-	-	-	-	-	Non-significativo= 0%
Media ponderada hBMMSC: +27,13% FS							
SHED	**LVDd (mm) Control 4 semanas**	**LVDd (mm) SHED 4 semanas**	**LVDs (mm) Control 4 semanas**	**LVDs (mm) SHED 4 semanas**	**FS% Control 4 semanas**	**FS% SHED 4 semanas**	**FS evolución %**
Petchdee *et al.*(19)	-	-	-	-	-	-	Non-significativo= 0%

Tabla 3. Resultados de la reducción del tamaño del infarto

	Tamaño del infarto Grupo de control después de 4 semanas (mm)	**Tamaño del infarto Grupo tratado después de 4 semanas (mm)**	**Evolución del tamaño del infarto %**
DPSCs			
Gandia *et al.* (24)	21,2 ± 1,6 %	15,9 ± 1,7 %	-25%
Reducción media ponderada del tamaño del infarto: - 25%			
SHED			
Petchdee *et al.*(19)	19,9 ± 0,03 %	10,9 ± 0,02 %	-45,2%
Reducción media ponderada del tamaño del infarto: - 45,2%			
hBMMSC			
Liu *et al.* (21)	54,9±3,3%	35,4±3,4%	-35,5%
Rasmussen *et al.* (23)	-	-	Non-significant = 0%
Shyu *et al.* (22)	66,1±1,5%	58,1±1,1%	-12,1%
Reducción media ponderada del tamaño del infarto: -18,67%			

Tabla 4. Resultados descriptivos de la evolución de la densidad vascular

	Grupo de control densidad vascular después de 4 semanas número de vasos por unidad de área (mm2)	**Densidad vascular del grupo tratado Después de 4 semanas Número de buques por unidad de área (mm2)**	**Evolución de la densidad vascular %**
DPSCs			
Gandia *et al.* (24)	518 ± 115/mm^2	868 ± 85/mm^2	+ 67,51%
Aumento medio ponderado de la densidad vascular: + 67,51%			
SHED			
Petchdee *et al.*(19)	-	-	Aumento del número de capilares basado en la tinción de H&E pero no cuantificado.
Aumento medio ponderado de la densidad vascular: No cuantificado			
hBMMSC			
Liu *et al.* (21)	144 ± 16/mm^2	938±42/mm^2	+84,64%
Rasmussen *et al.* (23)	-	-	No significativo = 0%
Shyu *et al.* (22)	439±27/mm^2	1119±17/mm^2	+60,8%
Aumento medio ponderado de la densidad vascular: + 34,57%			