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Angiogenesis Effects of Dental Stem Cells Cultured on Polymer Scaffolds

(Kesan Angiogenesis Sel Stem Pergigian yang Dibiakkan pada Perancah Polimer)

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ABSTRACT

Following the PRISMA-ScR guidelines, three electronic databases were searched (PubMed, Scopus and Web of Science) to identify the related studies using specific keywords and terms. The abstracts were evaluated for inclusion and exclusion criteria. The included publications were descriptively analysed. Out of 296 articles found, only nine were included for analysis. The objective of this study was to assess the angiogenesis effects of dental stem cells cultured in a polymer scaffold by evaluating their ability to promote blood vessel formation, cell viability, and tissue regeneration, thereby providing insights into their potential therapeutic applications in regenerative medicine. Previous studies mainly focused on polymer scaffold research, neglecting the crucial aspect of angiogenesis in pulp regeneration. Despite DPSCs' versatility in bone regeneration, more research is needed to understand their relationship with angiogenesis. The untapped potential of DPSCs in promoting blood vessel formation and tissue regeneration requires further exploration. Limited investigation exists on how the combination of stem cell, angiogenic, and dentin markers affects angiogenesis in DPSCs. The morphological changes DPSCs undergo in scaffold environments and the gene/protein expression analyses in DPSCs on scaffolds with angiogenic factors are areas that still need exploration. This research gap holds promise for enhanced understanding and advancement in tissue engineering and regenerative medicine, particularly in DPSCs' connection with scaffolds and angiogenesis. There has been limited research on the interplay of DPSCs, polymer scaffolds, and angiogenesis, with unexplored combined consequences on tissue regeneration. Scaffold-based techniques to investigate angiogenesis with DPSCs are uncommon. Further research might transform tissue engineering and regenerative medicine, spanning beyond dentistry.

Keywords: Angiogenesis; dental pulp stem cells; dentinogenesis; scaffold

ABSTRAK

Mengikut garis panduan PRISMA-ScR, tiga pangkalan data elektronik (PubMed, Scopus dan Web of Science) telah dicari untuk mengenal pasti kajian berkaitan menggunakan kata kunci dan istilah tertentu. Abstrak dinilai untuk kriteria kemasukan dan pengecualian. Penerbitan yang terpilih telah dianalisis secara deskriptif. Daripada 296 artikel yang diperoleh, hanya sembilan diterima untuk analisis. Objektif kajian ini adalah untuk menilai kesan angiogenesis sel stem pergigian yang dikultur dalam perancah polimer dengan menilai keupayaan mereka untuk menggalakkan pembentukan saluran darah, daya tahan sel dan penjanaan semula tisu, seterusnya memberikan pandangan tentang potensi aplikasi terapeutik mereka dalam perubatan regeneratif. Kajian terdahulu tertumpu terutamanya pada penyelidikan perancah polimer, mengabaikan aspek penting angiogenesis dalam penjanaan semula pulpa. Walaupun DPSC serba boleh dalam penjanaan semula tulang, lebih banyak penyelidikan diperlukan untuk memahami hubungan mereka dengan angiogenesis. Potensi DPSC yang belum diterokai dalam menggalakkan pembentukan saluran darah dan penjanaan semula tisu memerlukan penerokaan lanjut. Penyelidikan terhad wujud tentang bagaimana gabungan penanda sel stem, angiogenik dan dentin mempengaruhi angiogenesis dalam DPSC. Perubahan morfologi yang dialami DPSC dalam persekitaran perancah dan analisis ekspresi gen/protein dalam DPSC pada perancah dengan faktor angiogenik masih memerlukan penerokaan. Jurang penyelidikan ini

menjanjikan pemahaman dan kemajuan yang lebih baik dalam kejuruteraan tisu dan perubatan regeneratif, terutamanya dalam hubungan DPSC dengan perancah dan angiogenesis. Penyelidikan yang terhad mengenai interaksi DPSC, perancah polimer dan angiogenesis serta tiada penerokaan mengenai penggabungan semua faktor ini dalam penjanaan semula tisu. Teknik berasaskan perancah untuk mengkaji angiogenesis dengan DPSC jarang berlaku. Justeru, penyelidikan lanjut mungkin mengubah kejuruteraan tisu dan perubatan regeneratif pergigian.

Kata kunci: Angiogenesis; dentinogenesis; perancah; sel stem pulpa pergigian

INTRODUCTION

Angiogenesis is the process by which existing vasculatures expand into tissues, resulting in the formation of new blood vessels (Nur Syahidah et al. 2023). New vessels can be formed by sprouting or splitting existing vessels. Angiogenesis is a critical process in tissue repair and regeneration, as it ensures that the injured site is adequately supplied with immune cells, growth factors, and stem cells. Furthermore, angiogenesis leads to the formation of a vascular network that supplies oxygen and nutrients, and facilitates the clearance of metabolic waste products (Dudley & Griffioen 2023; Thomas, Manivasagan & Kim 2014). The angiogenesis process requires several endothelial cell responses, including proliferation and migration, which can be modulated by various cytokines (Nur Syahidah et al. 2023). In tissue healing processes, angiogenesis is involved in the proliferative phase. Proliferating cells regulate angiogenesis through several mechanisms. Proliferating cells release angiogenic factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), which promote endothelial cell growth and migration (Carmeliet & Jain 2011; Johnson & Wilgus 2014). Additionally, rapidly proliferating cells consume more oxygen, leading to increased hypoxic microenvironment. This hypoxic condition is a potent inducer of angiogenesis by stabilising and activating of hypoxia-inducible factors (HIF) in their microenvironment (Li, Zhao & Li 2021). Proliferating cells, especially in tissue remodelling or wound healing processes, can release matrix metalloproteinases (MMPs) (Kandhwal et al. 2022). MMPs help degrade the extracellular matrix, facilitating endothelial cell migration and promoting the sprouting and elongation of new blood vessels (Wang & Khalil 2018). Increased cell proliferation results in higher cell densities within tissues, enhancing direct interactions between proliferating cells and neighbouring endothelial cells, creating a conducive angiogenic microenvironment (Baptista et al. 2014; Tahergorabi & Khazaei 2012).

The processes of angiogenesis and dentinogenesis are closely related and interconnected during tooth development, repair, and regeneration (Saghiri et al. 2015). This process requires sufficient nutrients and oxygen to support the proliferation and differentiation of odontoblasts, the cells responsible for dentin formation (Baru et al. 2021; Demarco et al. 2011; Saghiri et al. 2015). Thus, proliferating dental pulp cells secrete angiogenic factors to promote angiogenesis, leading to the formation of an intricate network of blood vessels in pulp tissues. In cases of dental pulp tissue injury, angiogenesis in dental pulp tissues supports regenerative processes, helping to form reparative dentin to seal and protect the remaining vital pulp tissues (Soudi et al. 2021).

Dental pulp stem cells (DPSC) reside in the dental pulp tissues of permanent teeth. They exhibit a heterogeneous nature with characteristics of mesenchymal stem cells. DPSCs can differentiate into many cell lineages, including dentin-forming odontoblast-like cells, neural ectodermal cells and adipocytes, odontoblasts, osteoblasts, chondrocytes, and myoblast cells of mesodermal origin (Ledesma-Martínez, Mendoza-Núñez & Santiago-Osorio 2016; Sivadas, Rahul & Nair 2021). The less consideration of ethical issues against embryonic stem cells makes dental pulp stem cells an alternative source of stem cells used in regenerative treatment (Farinawati et al. 2018). DPSCs are the primary cells responsible for promoting dentin or pulp tissue regeneration after injury. DPSCs are found to reside around blood vessels in dental pulp tissues. In response to injury, cells around the blood vessels proliferate and migrate to the injury site. Subsequently, these perivascular cells differentiate into odontoblast-like cells and form reparative dentin (Mattei et al. 2021). This evidence implies the connection of DPSCs and vascular structures in physiological and pathological conditions.

Scaffolds, another critical component of tissue engineering, provide structural support for cell responses and subsequently lead to tissue healing and regeneration (Chan & Leong 2008). The vital roles played by scaffolds in engineered tissues are analogous to the functions of the extracellular matrix (ECM) in native tissues, such as providing structural support and biological guidance for cells to attach, grow, migrate, differentiate and respond to signals (Chan & Leong 2008). Numerous materials have been developed as scaffolds for tissue engineering applications. However, an ideal scaffold has yet to be achieved (Iannace, Sorrentino & Di Maio 2014). In addition to natural scaffolds, synthetic polymers have been introduced as alternative material sources for scaffold fabrication. There are advantages to synthetic polymer scaffolds, such as biocompatibility, versatility, and customizability. Synthetic polymer can be customised in terms of architecture and mechanical properties to meet the specific requirements of tissues engineering applications (Suamte et al. 2023). Synthetic polymer scaffolds can be designed to degrade at controlled rates (Guo & Ma 2014).

Many synthetic polymers used in tissue engineering are commercially available and cost-effective, making them accessible for clinical and research applications (Guo & Ma 2014). However, synthetic polymer scaffolds often lack the inherent biological cues presented in natural tissues (Iannace, Sorrentino & Di Maio 2014). Some synthetic polymers or their metabolites induce an inflammatory response upon implantation, which impedes the desired cellular interactions toward tissue regeneration (Chan & Leong 2008). Using synthetic polymer scaffolds in clinical applications requires regulatory approval, which can involve a rigorous evaluation and compliance with safety and efficacy standards (Chan & Leong 2008).

Previous research has shown the angiogenic capability of DPSCs and the impact of varying scaffolds on their behaviour. Nevertheless, there is still a need for an extensive understanding of the molecular and cellular mechanisms involved. For example, studies have shown that DPSCs possess the ability to secrete proangiogenic factors and promote the formation of blood vessels (Bar, Lis-Nawara & Piotr 2021; Hilkens et al. 2013). However, the signalling mechanisms and growth factors influencing angiogenesis in this scenario are still unclear. The insufficient understanding of the ideal conditions and scaffold compositions to optimize the angiogenic potential of DPSCs represents a major knowledge gap. Most research has concentrated on the broad usage of polymer scaffolds without carefully examining how various scaffold attributes, such as mechanical strength, porosity, and biodegradability, affect angiogenesis. Comprehensive research examining the long-term impacts of scaffolds seeded with DPSCs on tissue regeneration and functional recovery is also lacking. Additionally, there has been insufficient investigation into the molecular mechanisms underlying the interaction between polymer scaffolds and DPSCs, which is essential for maximizing their combined therapeutic potential. Identifying and characterising these variables could provide critical insights into angiogenic processes and enable the development of more tailored techniques to increase angiogenesis in stem cell-based dental treatments. While some studies have investigated the impact of scaffold properties such as material composition, porosity, and surface topography on cell behaviour (Deng et al. 2020; Diana et al. 2020). The role of scaffold composition and architecture in inducing angiogenesis by dental stem cells needs to be clarified. Addressing these knowledge gaps will lead to a better understanding of the angiogenic effect on the growth of dental pulp stem cells within polymer scaffolds, resulting in more effective regenerative strategies for dentistry. The present study aims to review and discuss the angiogenesis effects of dental pulp stem cells cultured on polymer scaffolds.

METHODS

SEARCH STRATEGY

The Joanna Briggs Institute guideline for scoping reviews (Peters et al. 2020), in tandem with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis extension for scoping reviews (PRISMA-ScR) (Tricco et al. 2018) was employed. The research question for this scoping review was "How do angiogenesis features of dental pulp stem cells affect cellular behaviour cultured within polymer scaffold in terms of the ability to differentiate into endothelial cells and its relationship with angiogenic factors?" The literature was searched in PubMed, Scopus, and Web of Science databases from the earliest available database until March 2023. The search terms were ((‘Dental Pulp Stem Cells’) OR ‘(Scaffolds’ OR ‘Biomaterials’) AND (‘Angiogenesis’)). The language was limited to English language only. The publications were screened for inclusion criteria and the bibliographies of the selected studies were screened for other relevant studies. Scopus - (TITLE-ABS-KEY (dental AND pulp AND stem AND cells) OR TITLE-ABS-KEY (scaffold OR biomaterials) AND TITLE-ABS-KEY (angiogenesis)).

CRITERIA FOR STUDY SELECTION

The inclusion criteria for the selection of articles were defined according to the participants, concept, and context domain (PCC): Participants (P): *In vitro* dental stem cell culture; Concept (C): All dental pulp stem cells cultured in polymer scaffolds related to angiogenesis mechanism; Context (C): Types of cells used, biomarkers, and method of analysis. Publications that were review papers, notes, letters, book chapters, and case studies were excluded. Research articles that investigated other areas, such as the implant, tumour, and animal studies, were also excluded. Subsequently, the research articles were screened on titles and abstracts for related terms to the angiogenesis mechanism of DPSCs.

DATA EXTRACTION

A total of 296 unique articles were initially identified. Two independent reviewers (NHD and FY) identified titles and abstracts. Twenty-three duplicates of publications and twenty-seven off-type articles were excluded. Disagreements between reviewers were resolved by discussion until a consensus was reached, or with a third reviewer (NMA). A total of 264 potentially eligible full articles were considered in the full-test screening according to the inclusion and exclusion criteria. After full text evaluation, 255 were excluded because the

publications were off-topic articles and did not meet the inclusion criteria. A total of 9 articles were included in the final review. Table 1 shows an example of the template for extraction information that we used. A summary of the studies' screening process is presented in Figure 1.

Data from the selected studies were extracted by two reviewers independently using a result extraction instrument indicated by the methodology for scoping reviews developed by the Joanna Briggs Institute (Peters et al. 2020). Both reviewers independently used the data extraction instrument to extract data from the first five studies and discussed whether their approach was consistent with the research questions and objectives (Levac, Colquhoun & O'Brien 2010). Any disagreements that arose between the reviewers were resolved by discussion. A third review author was consulted as needed. Data were tabulated in Microsoft Excel 365 according to the publication details (authors and year), types of scaffolds, biomarkers, and analysis methods. The methodological quality and risk of bias of the included studies were not appraised since it is not relevant for a scoping review. However, some limitations of the studies were noted and reported at the end of this review to provide valuable information for future research studies or systematic reviews.

RESULTS

STUDY CHARACTERISTICS

The main characteristics of the included studies are described in Tables 2, Table 3 and, Table 4. The publications were grouped based on types of scaffolds, biomarkers, and analysis methods related to DPSCs. A total of nine publications employed DPSCs (Cavalcanti, Zeitlin & Nör 2013; Dissanayaka et al. 2015; Divband et al. 2022; Galler et al. 2012; Tien et al. 2021; Xia et al. 2020; Zhang et al. 2017; Zhang et al. 2021).

Table 2 summarizes the types of scaffolds used in various research studies, indicating whether each study employed synthetic or natural scaffolds. It lists the authors and publication years of the studies, showing a predominance of synthetic scaffold use across different years. Specifically, out of the nine studies presented, seven used synthetic scaffolds, while only two used natural scaffolds.

Table 3 shows the biomarkers analyzed in each study. Each row lists the authors and the year of publication, followed by the specific biomarkers analyzed in the respective study. These biomarkers are associated with various biological processes, including cell proliferation, angiogenesis, and tissue differentiation. The table shows a diverse range of biomarkers utilized across the studies, reflecting the complexity and breadth of research in tissue engineering. By highlighting these biomarkers, the table underscores the multifaceted approaches researchers employ to investigate and understand the underlying mechanisms in scaffold-based tissue regeneration.

Table 4 shows the analysis method employed. This table details the methodologies used in various research studies to analyze morphology, cell markers, angiogenesis activity, cell proliferation, and gene and protein expression. Each row lists the authors and the year of publication, followed by the specific methods employed. Techniques such as flow cytometry, scanning electron microscopy (SEM), immunofluorescence (IF), confocal microscopy, and RT-PCR are commonly used across these studies. Additionally, assays like MTT, DAPI staining, ELISA, and various angiogenesis and differentiation assays are utilized to investigate biological processes, including cell proliferation, angiogenesis, and tissue differentiation. The diverse range of methods reflects the comprehensive approaches taken by researchers to explore the complexities of scaffold-based tissue engineering.

TYPES OF SCAFFOLDS

The types of scaffolds used in the selected papers were divided into synthetic and natural polymer scaffolds. The synthetic polymer scaffolds used were synthetic biodegradable scaffold (Zhang et al. 2021), CA-coated MTA (CAMTA) scaffold (Tien et al. 2021), self-assembling peptides (SAPs)-based scaffold (Xia et al. 2020), calcium phosphate cement (CPC) scaffold (Zhang et al. 2017), peptide hydrogel PuraMatrix™ scaffold (Dissanayaka et al. 2015), self-assembling peptide nanofibers (MDPs) hydrogel scaffold (Galler et al. 2012), and Puramatrix™ self-assembling peptides (SAPs) hydrogel scaffold (Cavalcanti, Zeitlin & Nör 2013). On the other hand, the natural synthetic scaffolds were chitosan polymeric scaffolds (Divband et al. 2022) and biocoral scaffolds (Mangano et al. 2011). The most represented was synthetic polymer scaffolds, with a total of seven studies using synthetic polymer scaffolds.

BIOMARKER

The selected papers showed significant variations concerning the types of biomarkers, stem cell, angiogenic, and dentin markers. Seven papers reported the use of stem cell markers, angiogenic markers, and dentin markers, respectively. The papers reported the stem cell markers were Dissanayaka et al. (2015), Galler et al. (2012), Xia et al. (2020), Zhang et al. (2017), and Zhang et al. (2021). On the other hand, the papers that reported the angiogenic markers were Divband et al. (2022), Tien et al. (2021), Xia et al. (2020), Zhang et al. (2017), and Zhang et al. (2021). The papers that reported the dentin markers were Cavalcanti, Zeitlin and Nör (2013), Dissanayaka et al. (2015), Mangano et al. (2011), Tien et al. (2021), Zhang et al. (2017) and Zhang et al. (2021).

TABLE 1. Template for extraction information based on inclusion and exclusion criteria

Data point	Extracted information
Databased searching	PubMed
Year of publication	2021
Type of paper	Original manuscript
Full text article assessed	Yes
Study design	*In vitro*
Language	English
Related to angiogenesis	Yes

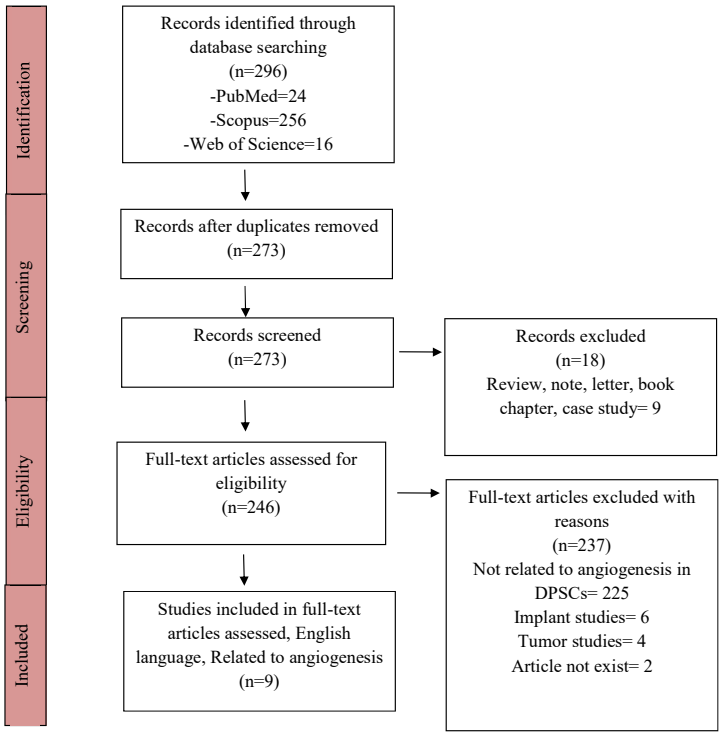

FIGURE 2. Flowchart for study screening and selection (n=number of articles)

TABLE 1. Type of scaffold used

Author/Year	Type of scaffolds
Zhang et al. (2021)	Synthetic
Divband et al. (2022)	Natural
Mangano et al. (2011)	Natural
Tien et al. (2021)	Synthetic
Xia et al. (2020)	Synthetic
Zhang et al. (2017)	Synthetic
Dissanayaka et al. (2015)	Synthetic
Galler et al. (2012)	Synthetic
Cavalcanti, Zeitlin & Nör (2013)	Synthetic

TABLE 2. Biomarkers analyzed in each study

Author/Year	Biomarkers	
Zhang et al. (2021)	Stem cell	p53/p21
		BMI-1
		CD44
		CD73
		CD90
		CD105
	Angiogenic	VEGFR-2
		Tie-2
		CD31
		VE-cadherin
		DPSC-GFP
	Dentin	DSPP
		DMP-1
Divband et al. (2022)	Angiogenic	VEGFR-2
		Tie-2
		Ang-1
		PDGF
		bFGF
		TGF
		VEGF
Mangano et al. (2011)	Dentin	Osterix
		Runx-2
		Osteocalcin
		Osteonectin
		BMP-2
		VEGF
		BAP
		OPN
		BSP
Tien et al. (2021)	Angiogenic	VEGF
	Dentin	Ang-1
		Ca
		Si
		Hydroxyl
		ALP
		BSP

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Xia et al. (2020)	Stem cell	CD105
		CD90
		CD34
		CD45
	Angiogenic	VEGF
		CD31
		Ang-1
		vWF
	Dentin	ALP
		DMP-1
		DSSP
Zhang et al. (2017)	Stem cell	CD90
		CD105
	Angiogenic	PDGF-BB
	Dentin	DMP-1
		DSSP
		CD90
Dissanayaka (2015)	Stem cell	STRO-1
		CD73
		CD105
		CD90
	Dentin	ALP
Galler et al. (2012)	Stem cell	bFGF
		TGFβ1
		VEGF
Cavalcanti, Zeitlin & Nör (2013)	Dentin	DSSP
		DMP-1

BMI-1, B cell-specific Moloney murine leukemia virus integration site 1; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor 2; PDGF, Platelet-derived growth factor; FGF, Basic fibroblast growth factor; TGF, transforming growth factor; TGFβ1, transforming growth factor beta 1; PDGF-BB, platelet-derived growth factor subunit B; Ang-1, Angiopoietin-1; Tie-2, Angiopoietin-1 receptor; VE-cadherin, Vascular endothelial cadherin; DPSC-GFP, dental pulp stem cell-green fluorescent protein; DSPP, dentin sialophosphoprotein; DMP-1, dentin matrix protein 1; BMP-2, bone morphogenetic proteins-2; BAP, bone-specific alkaline phosphatase; OPN, Osteopontin; BSP, bone sialoprotein; STRO-1, stromal cell precursor surface antigen 1; Runx-2, Runt-related transcription factor 2; Ca, calcium ion; Si, silicon ; ALP, alkaline phosphatase; vWF, von willebrand factor

TABLE 3. Analysis method employed

Author/Year	Method	
Zhang et al. (2021)	Morphology and cell markers	Flow cytometry
	Angiogenesis activity	GFP
		Immunofluorescence
		In vitro capillary-tube assay
	Genes and proteins expression	RT-PCR analyses with p53-silenced DPSC
		Western blots
Divband et al. (2022)	Morphology and cell markers	SEM
	Proliferation of the cells	SEM
		MTT assay
		DAPI staining
	Genes and proteins expression	RT- PCR
		Western blot
Mangano et al. (2011)	Morphology and cell markers	SEM
		Toluidine blue staining
	Angiogenesis activity	ELISA
	Genes and proteins expression	RT-PCR
	Dentinogenesis	Alizarin red staining
Tien et al. (2021)	Morphology and cell markers	EZ Test machine
		Confocal microscope
	Angiogenesis activity	ELISA
		Angiogenesis assay
		Osteogenesis assay
	Proliferation of the cells	Confocal microscope
	Dentinogenesis	µCT
Xia et al. (2020)	Morphology and cell markers	IF staining
		FTIR
	Angiogenesis activity	Tube formation
	Proliferation of the cells	Confocal microscope
		Cell proliferation assay
Zhang et al. (2017)	Morphology and cell markers	SEM
	Angiogenesis activity	Immunofluorescence
		In vitro angiogenesis
	Proliferation of the cells	Inverted fluorescence microscopy
		Flow cytometry
		MTT assay
	Genes and proteins expression	RT-qPCR
		Western blot
	Dentinogenesis	Cell Differentiation Analysis

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Dissanayaka et al. (2015)	Morphology and cell markers	Transwell assay
		Confocal microscopy
	Angiogenesis activity	ELISA
		In vitro prevascularization
	Proliferation of the cells	Live/Dead viability assay
		Transfection of cells with green fluorescent protein and red fluorescent protein constructs
		Osteogenic differentiation assays
	Dentinogenesis	ALP
Galler et al. (2012)	Morphology and cell markers	Confocal microscopy
	Angiogenesis activity	ELISA
Cavalcanti, Zeitlin & Nör (2013)	Morphology and cell markers	Confocal microscopy
	Proliferation of the cells	WST-1 Assay
	Genes and proteins expression	RT-PCR

GFP, Green fluorescence protein; SEM, scanning electron microscopy; RT-PCR, real-time reverse transcription–polymerase chain reaction; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DAPI, 4′,6-diamidino-2-phenylindole; µCT, micro-computed tomography; IF, immunofluorescence; ELISA, enzyme-linked immunosorbent assay; FTIR, fourier transform infrared spectroscopy; ALP, alkaline phosphatase; WST-1, water-soluble tetrazolium salt-1

METHODS OF ANALYSIS

Most of the papers focused on analysing the method used to study cell morphology, with a total of 9 articles (Cavalcanti, Zeitlin & Nör 2013; Dissanayaka et al. 2015; Divband et al. 2022; Galler et al. 2012; Mangano et al. 2011; Tien et al. 2021; Xia et al. 2020; Zhang et al. 2017; Zhang et al. 2021). Six papers that analysed the method for angiogenesis activity (Dissanayaka et al. 2015; Galler et al. 2012; Tien et al. 2021; Xia et al. 2020; Zhang et al. 2017; Zhang et al. 2021). The papers which analysed the method for proliferation activity also consist of a total of six articles (Cavalcanti, Zeitlin & Nör 2013; Dissanayaka et al. 2015; Divband et al. 2022; Tien et al. 2021; Xia et al. 2020; Zhang et al. 2017). The method of analysis of genes and proteins has been reported by five papers (Cavalcanti, Zeitlin & Nör 2013; Divband et al. 2022; Mangano et al. 2011; Zhang et al. 2017; Zhang et al. 2021). Lastly, four papers reported the method analysis of dentinogenesis (Dissanayaka et al. 2015; Mangano et al. 2011; Tien et al. 2021; Zhang et al. 2017).

DISCUSSION

TYPES OF SCAFFOLDS AND THEIR CHARACTERISTICS

Both natural and synthetic polymer scaffolds have benefits and limitations, and the choice is influenced by various of factors. Natural scaffolds are typically derived from biological sources such as extracellular matrix (ECM) components or biomaterials from living organisms. These scaffolds provide a supportive environment for cells to attach, proliferate, and differentiate, which are essential for tissue regeneration (Chan & Leong 2008). In the context of angiogenesis, natural scaffolds can help promote this process in several ways, such as mimicking the ECM. Natural scaffolds often contain components similar to the ECM of native tissues (Mastrullo et al. 2020). These components, such as collagen, fibronectin, and laminin provide signalling cues that guide endothelial cells to migrate, proliferate, and form new blood vessels (Khanna, Zamani & Huang 2021). Furthermore, natural scaffolds can be engineered to sequester and release growth factors that are involved in angiogenesis, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). These growth factors stimulate endothelial cell proliferation and migration, thereby promoting blood vessel formation (Mastrullo et al. 2020). Natural scaffolds can also support the incorporation of various cell types, including endothelial cells and pericytes. These cells play crucial roles in angiogenesis by forming the inner lining and supporting the walls of blood vessels, respectively (Thottappillil & Nair 2015).

Synthetic scaffolds are designed and manufactured using synthetic materials, often synthetic polymers or ceramics, which can be tailored to have specific properties. Synthetic scaffolds can be engineered to have specific mechanical, chemical, and degradation properties that influence cell behaviour, including angiogenesis (Suamte et al. 2023). In addition, synthetic scaffolds offer greater versatility in terms of design and fabrication, enabling

researchers to create structures with defined shapes, sizes, and architectures that are optimised for enhancing angiogenesis (Suamte et al. 2023). In this regard, scaffold stiffness, porosity, and degradation rate can impact endothelial cell recruitment and behaviour (Mastrullo et al. 2020). Synthetic scaffolds can also be functionalised with bioactive molecules, such as growth factors or peptides, to enhance angiogenic responses. Controlled release of these molecules from the scaffold can provide sustained cues for endothelial cell activity (Marwa et al. 2015).

In all of the included papers, they conducted studies using polymer scaffolds, but it is difficult to find any articles reported on angiogenesis, which is essential in pulp regeneration. Many studies exploring angiogenesis utilise human umbilical vein endothelial cells (HUVEC) rather than DPSCs. These studies likely aimed to better understand the mechanisms and factors that drive the formation of new blood vessels, as HUVEC is commonly used as a model for angiogenesis research. Despite the existing research, there is still a significant gap in investigating the interaction between DPSCs, polymer scaffolds, and the crucial process of angiogenesis. This highlights the need for further exploration in this particular field such as DPSC interaction with polymer scaffolds, specifically focusing on their role in angiogenesis. More comprehensive research is necessary to fully understand how DPSCs interact with specific scaffold materials and how this interaction influences angiogenesis. Enhancing our understanding of these interactions holds great potential for advancing tissue engineering and regenerative medicine applications, not only in the realm of dentistry but also in broader medical contexts. By delving deeper into the complex relationship between stem cells, scaffold materials, and the intricate process of angiogenesis, we can potentially develop more effective strategies for tissue repair and regeneration strategies with implications that extend beyond dentistry and into various medical disciplines.

BIOMARKERS

All included articles showed a positive result for all biomarkers, and the cell morphology of the dental stem cells involved. A positive result for the stem cell markers, in combination with the usual morphology of a dental tissue sample, would indicate and confirm the presence of cells.

STEM CELL MARKERS

Stem cell markers are usually specific proteins or molecules expressed on the surface or within stem cells. These markers are used to recognise and identify stem cells that were isolated from tissues (Hagar et al. 2021) crucially distinguishing stem cells from other cell types and understanding their properties (Pazhanisamy 2013). Stem cells can be identified and isolated based on expression of specific proteins on their surface. Commonly used surface markers include CD34, CD44, CD133, CD90, CD105, and SSEA-4 (Kim & Ryu 2017; Lv et al. 2017). For example, CD34 is often used as a marker for hematopoietic stem cells, while CD44 is associated with mesenchymal stem cells (Sidney et al. 2014). Besides the surface protein markers, the transcription factors can also be used. Transcription factors are proteins that regulate gene expression and play a crucial role in biological responses, including stem cell maintenance and differentiation (Ahmed et al. 2009; Islam et al. 2021). Examples of transcription factors used as pluripotent stem cell markers include OCT_4, NANOG, SOX_2, and KLF_4. These factors are associated with pluripotency and self-renewal of embryonic stem cells (Kashyap et al. 2009; Swain et al. 2020).

Stem cells respond to specific signalling pathways that regulate their self-renewal and differentiation. Activation or repression of these pathways can be used as markers to identify stem cells (Tanabe 2015). Examples include the Wnt signalling pathway, Notch signalling pathway, and Hedgehog signalling pathway. These signalling play essential roles in stem cell maintenance and fate determination (Kumar et al. 2021). Stem cells can be characterised based on the expression of markers associated with specific lineages or differentiation stages. These markers indicate the commitment of stem cells to a particular cell lineage, implying the reduction of stemness properties (Nguyen, Nag & Wu 2010; Zakrzewski et al. 2019).

ANGIOGENIC MARKERS

Angiogenesis markers are specific proteins or molecules that play a role in the formation of new blood vessels from pre-existing ones. These markers are expressed during the process of angiogenesis (Johnson & Wilgus 2014). Vascular endothelial growth factor (VEGF) is one of the most well-known angiogenesis markers and plays a crucial role in promoting the formation of new blood vessels. It stimulates endothelial cell proliferation, migration, and tube formation. VEGF isoforms, particularly VEGF-A, are commonly used as markers to evaluate angiogenic activity formation (Niu & Chen 2010; Tahergorabi & Khazaei 2012). Fibroblast growth factors (FGF) are a family of growth factors involved in angiogenesis. FGF2 is widely used as a marker to assess angiogenic potential and evaluate angiogenesis-related diseases (Cao et al. 2004; Jia et al. 2021). Platelet-derived growth factor (PDGF) is a growth factor that contributes to angiogenesis by promoting the recruitment and proliferation of pericytes and smooth muscle cells (Andrae, Gallini & Christer 2008;

Raica & Cimpean 2010). PDGF isoforms, particularly PDGF-BB, play a role in stabilising newly formed blood vessels. Angiopoietins are a group of growth factors involved in the regulation of angiogenesis. Angiopoietin-1 (Ang-1) promotes stabilisation and maturation of blood vessels, while angiopoietin-2 (Ang-2) acts as either a pro- or antiangiogenic factor, depending on the context. Angiopoietin-2 is often used as a marker of endothelial cell activation and remodelling during angiogenesis (Akwii et al. 2019; Olver, Ferguson & Laughlin 2015). Matrix metalloproteinases (MMPs) are a family of enzymes that play a crucial role in extracellular matrix remodelling during angiogenesis (Cabral-Pacheco et al. 2020). Certain MMPs, such as MMP-2 and MMP-9, are involved in the breakdown of the extracellular matrix, allowing endothelial cells to migrate and form new blood vessels (Cabral-Pacheco et al. 2020; Wang & Khalil 2018). MMPs are also used as markers to evaluate the proteolytic activity associated with angiogenesis. These angiogenesis markers provide valuable information on the mechanisms and regulation of blood vessel formation. Their expression levels and activity can be used to evaluate angiogenic processes in various physiological and pathological conditions I (Quintero-Fabián et al. 2019; Roy, Yang & Moses 2019).

DENTIN MARKERS

Dentin markers are specific proteins or molecules that are expressed during the formation and mineralisation of dentin, a hard tissue that makes up the bulk of the tooth structure. These markers are used to identify and characterise the different stages of dentin formation (Galler et al. 2012; Golberg et al. 2011). Dentin sialophosphoprotein (DSPP) is a key marker of dentinogenesis and is involved in dentin matrix formation and mineralisation. DSPP undergoes proteolytic processing, resulting in the generation of dentin sialoprotein (DSP) and dentin phosphoprotein (DPP), both of which contribute to dentin structure and mineralisation (Prasad, Butler & Qin 2010; Yamakoshi 2009). DSPP mutations are associated with various dentin disorders, highlighting their importance in dentinogenesis (Yamakoshi 2008).

Dentin matrix protein 1 (DMP1) is a noncollagenous protein that plays a critical role in dentin mineralisation. It regulates the formation and maturation of dentin by interacting with calcium ions and promoting hydroxyapatite crystal growth (Prasad, Butler & Qin 2010; Ravindran & George 2015; Suzuki et al. 2012). DMP1 and DMP2 are the two main isoforms present in dentin, and DMP1 is predominantly associated with dentin mineralisation (Golberg et al. 2011; Suzuki et al. 2012). DMP1 mutations are associated with dentin defects and can result in abnormal dentin formation (Yamakoshi 2008). Dentin glycoprotein (DGP) is a family of glycoproteins expressed in dentin and is involved in dentinogenesis. Dentin glycoproteins contribute to the organisation and mineralisation of the dentin matrix mineralisation (Golberg et al. 2011; Yamakoshi 2009). Dentin sialoprotein (DSP) and dentin matrix protein 2 (DMP2) are examples of dentin glycoproteins that play crucial roles in dentin mineralisation. These dentin markers help to understand the molecular processes underlying dentin formation and can contribute to the diagnosing and characterising of dentin-related disorders (Suzuki et al. 2012; Yamakoshi 2008).

DPSCs, being a reservoir of cells capable of differentiating into dentin-forming cells known as odontoblasts, express these dentin markers (La Noce et al. 2014). While the direct relationship between dentin markers and angiogenesis is less established, it is plausible that DPSCs, with their potential to differentiate into multiple lineages, could indirectly impact angiogenesis during tissue regeneration (Mattei et al. 2021). As DPSCs differentiate into odontoblast-like cells to form dentin, they can also release signals that influence nearby endothelial cells, potentially affecting angiogenesis in the dental pulp microenvironment (Marrelli et al. 2018).

To conclude, studies have explored the relationship between stem cell markers, angiogenic markers, dentin markers, and the angiogenesis process using DPSCs, but are very limited. DPSCs have a remarkable ability to differentiate into multiple cell lineages, including endothelial cells, which are required for blood vessel formation, and dentin-forming cells, which are necessary for dentin regeneration. The intersection of these disparate activities creates an interesting issue in which stem cell indicators direct DPSCs behaviour, angiogenic markers direct the development of new blood vessels, and dentin markers direct the rebuilding of reparative dentin. The current limited study of the association between these markers and the angiogenesis process using DPSCs just hints at the extensive terrain that needs to be explored further.

METHODS OF ANALYSIS

CELLULAR MORPHOLOGY

Cell morphology refers to the study of the structure and shape of cells, including their size, appearance, and internal organisation (Weinhardt et al. 2019). Observing and characterising cell morphology can provide valuable insights into cell function, differentiation, and health (Mazzarini et al. 2020). Cell morphology can be examined using various techniques, for example, flow cytometry, light microscope, electron microscope, immunofluorescence, and confocal microscope (Alfonso & Al-Rubeai 2011; Alvarez-Barrientos et al. 2000; McKinnon 2018).

Understanding how DPSCs contribute to blood vessel development and tissue regeneration requires a method to analyse cell morphology in relation to DPSCs and the angiogenesis process. Examining cell morphology provides

beneficial details on the structural and functional changes that occur in DPSCs during angiogenesis. These changes can include changes in cell shape, cytoskeletal rearrangements, and the formation of cellular extensions, which are indicative of cell migration and interaction with other cells (Lamalice, Le Boeuf & Huot 2007). Morphological analysis allows researchers to quantify various cellular features that are associated with angiogenesis. These features could include the extension of cellular processes such as lamellipodia and filopodia, the alignment of cells in the direction of blood vessel growth, and the organisation of cells into tube-like structures mimicking blood vessels (Gerhardt et al. 2000; Lamalice, Le Boeuf & Huot 2007). Quantitative data on these morphological parameters can provide insights into the angiogenic potential of DPSCs (Dissanayaka et al. 2015). DPSCs differentiate into endothelial-like cells and play a vital role in angiogenesis. Monitoring changes in cell morphology can indicate whether DPSCs are adopting a more endothelial-like appearance, characterised by elongated and interconnected cell shapes (Singh et al. 2018). This differentiation is often accompanied by changes in cell adhesion molecules and cytoskeletal elements, which can be assessed through morphological analysis (Luo et al. 2022).

The methods mentioned thus far have been used to reliably identify and define certain cell types under investigation. Conspicuously missing from the existing body of research is an analysis of the methods that have been used to validate and elucidate the intricate morphological properties of dental pulp stem cells (DPSCs) when cultured with scaffolds, particularly in the context of angiogenic factors. In essence, while studies have diligently employed methods to ensure the accurate identification of the cell populations being studied, there appears to be a significant gap in terms of investigations aimed at elucidating the visual and structural aspects of DPSCs' behaviour when exposed to scaffolds and angiogenic factor cues. This gap represents uncharted territory in which researchers have yet to investigate the subtle but critical changes in cell shape, arrangement, and interactions that DPSCs undergo when influenced by the three-dimensional environment provided by scaffolds and in the presence of angiogenesis-promoting factors. Morphology, which is frequently closely related to cell function, is crucial in understanding how DPSCs interact with their environment and contribute to processes such as angiogenesis. The scarcity of studies on these morphological alterations, strong demand more research. Understanding the physical manifestations of DPSC responses to scaffolds and angiogenic factors, such as the extension of cellular processes, alignment, or the formation of vessel-like structures, can provide invaluable insights into the intricate ballet of cell behaviour that supports tissue regeneration and the formation of new blood vessels.

ANGIOGENESIS

Assessing angiogenesis activity is essential in research and clinical settings to understand disease progression and evaluate the efficacy of angiogenesis-targeted therapies (Niu & Chen 2010; Tahergorabi & Khazaei 2012). Several methods can be used to assess angiogenesis activity. Examples of the *in vivo* assay are the matrigel assay and the corneal angiogenesis assay. In the matrigel assay, growth factor-enriched matrigel, which is a gelatinous basement membrane matrix, is injected subcutaneously into experimental animals, such as mice (Antonino, Kleinman & Martin 2021; Ponce 2001). The corneal angiogenesis assay involves placing an angiogenic stimulus, such as a pellet containing angiogenic factors, onto the cornea of an animal (Azar 2016).

The aortic ring assay is categorised as an *ex vivo* assay. In this assay, rings from the aortas of animals are embedded in a gel matrix and cultured *ex vivo*. Endothelial cells from the aortic rings migrate and form new vessel-like structures, which can be quantified and analysed after a period of culture (Azar 2016). In addition, an endothelial cell tube formation assay is widely used *in vitro* assay involves culturing endothelial cells on a suitable matrix, such as matrigel or collagen. Under the influence of angiogenic factors, endothelial cells form tube-like structures, mimicking blood vessels (DeCicco-Skinner et al. 2014; Kelley et al. 2022). In addition to that, the endothelial cell migration assay can be used to measure the migration of endothelial cells in response to angiogenic stimuli. Cells are seeded on the surface and a gradient of angiogenic factors is created to induce cell migration. The number of migrated cells is quantified after a specific time (Staton, Reed & Brown 2009; Tahergorabi & Khazaei 2012). Another indirect approach for angiogenesis evaluation is to determine the expression of angiogenesis-related growth factors. The levels of pro-angiogenic factors, such as VEGF or FGF, can be measured in blood or tissue samples using techniques such as ELISA or immunohistochemistry (Kennedy, Wheatley & McCullagh 2022; Tahergorabi & Khazaei 2012).

However, studies rarely look for angiogenesis by using dental pulp stem cells cultured with scaffolds. Instead, a popular method for researching angiogenesis-related processes has been the using matrigel. While matrigel provides a supportive environment to examine angiogenic responses, the use of scaffolds with regulated physical and chemical characteristics have yet to be studied as thoroughly in this context. In light of this, more study is essential on the particular relationships between DPSCs, scaffolds, and angiogenesis. Investigating angiogenesis in the context of scaffolds might provide insight into the intricate interaction of stem cells, the physical environment, and the biochemical signals that drive the development of blood vessels.

GENE AND PROTEIN EXPRESSION

Gene and protein expression analyses are crucial techniques in Molecular Biology and Biomedical Research (Singh et al. 2018). Several methods are used to analyse gene expression, such as conventional reverse transcription polymerase chain reaction (RT-PCR), quantitative RT-PCR, microarrays, and RNA sequencing. RT-PCR is a widely used technique to measure the mRNA level produced from specific genes with semi-quantitative evaluation, while quantitative RT-PCR (qPCR) allows real-time monitoring of PCR product accumulation. The qPCR provides a more accurate and quantitative measurement of gene expression levels by using fluorescent dyes or probes that bind to PCR products during amplification (Mo, Wan & Zhang 2012). RNA sequencing (RNA-seq) is also a powerful method providing comprehensive and unbiased analysis of gene expression analysis. It involves sequencing the entire transcriptome to identify and quantify mRNA levels, including known and novel transcripts (Wang, Gerstein & Snyder 2009). Lastly, microarrays are a method that uses a collection of DNA or RNA probes immobilised on a solid surface to detect and quantify gene expression. They can simultaneously analyse the expression of thousands of genes in a single experiment (Rashid, Husnain & Riazuddin 2014).

For protein expression analysis, western blotting is a commonly used technique to analyse the specific protein expression (Mahmood & Yang 2012). Furthermore, an enzyme-linked immunosorbent assay (ELISA) is a quantitative method used to measure the concentration of a particular protein in a sample. It involves immobilising an antigen-specific antibody on a solid surface and using another antibody linked to an enzyme to detect the target protein (Sakamoto et al. 2017). However, immunohistochemistry (IHC) is used to visualize the localisation and expression of proteins in tissue samples. Specific antibodies are used to stain tissue sections, and the presence and distribution of the protein of interest are observed under a microscope (Magaki et al. 2018). Flow cytometry is a method that can be used to analyse protein expression in individual cells within a heterogeneous population. Cells are labelled with fluorescently conjugated antibodies targeting specific proteins, and their fluorescence is measured as they pass through a flow cytometer (McKinnon 2018). Proteomic analysis can be also utilised to determine protein expression profiles using the mass spectrometry technique.

These methodologies have primarily focused on confirming the correctness of gene and protein expression in the context of angiogenesis investigation. However, there appears to be a noteworthy gap in the present corpus of research, such as a shortage of studies that look into the utilisation of these methodologies, especially for DPSCs cultivated on scaffolds and their interaction with angiogenic factors. The approaches to validate gene and protein expression are powerful tools to unravel the molecular complexities that regulate angiogenesis. These tools enable researchers to determine the genetic sequences and protein markers that regulate cell activity, especially when driving processes such as blood vessel development. However, the absence of publications evaluating the application of these approaches to DPSCs grown in scaffold settings under the impact of angiogenic factors suggests an unexplored area of research. Researchers can gain new insights by studying gene and protein expression in the context of DPSCs, scaffolds, and angiogenic stimuli. Researchers may possibly identify the complicated methods by which DPSCs contribute to angiogenesis in the context of tissue regeneration by merging gene and protein expression investigations with scaffold-based DPSC cultures and angiogenic agents. Overall, the lack of studies that combine gene and protein expression analysis with scaffold-based DPSCs and angiogenic agents presents an exciting route for future studies.

DENTINOGENESIS

Dentinogenesis analysis studies the formation and development of dentin, the hard tissue that makes up most of the tooth (Goldberg et al. 2011). Dentinogenesis is a critical process in tooth development, repair, and response to various dental treatments. Dentinogenesis analysis is essential to understanding tooth development, the repair process in dental treatments such as cavity restoration, and the response of dentin to different dental materials and interventions (Smith & Sharpe 2014). For studying dentinogenesis, tooth samples are collected, fixed, and processed to obtain thin sections. These sections are stained with specific dyes, such as hematoxylin and eosin (H&E), to visualise the different stages of dentin formation. Immunohistochemical techniques involve the use of specific antibodies to target and detect proteins involved in dentinogenesis, such as dentin sialoprotein (DSP) or dentin matrix protein 1 (DMP-1) (Paula et al. 2019). Scanning electron microscopy (SEM) can be used to visualise the detailed surface morphology of dentin and its mineralisation patterns. It provides high-resolution images, helping to study dentin structure and the organisation of dentinal tubules (Kuntze et al. 2020). RT-PCR and qPCR are the techniques used to analyse the expression of genes involved in dentinogenesis, such as dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP-1), and alkaline phosphatase (ALP) (Huang et al. 2019). Dental pulp-derived cells, such as odontoblasts, can be cultured *in vitro* to study odontogenic differentiation under controlled conditions. Various factors, such as growth factors and extracellular matrix components, can be added to the culture to investigate their role in dentin formation (Mortada & Mortada 2018). For example, the direct interaction of VEGF, which is a growth factor, shows increased proliferation and differentiation with the receptors expressed by the osteogenic cells (Tsai et al. 2023).

Although these methodologies have been used systematically to investigate the molecular and cellular complexities underlying dentinogenesis, their applicability to the interaction between DPSCs, scaffolds, and the complicated arrangement of angiogenesis still needs to be discovered. This previously unexplored area offers an unparalleled opportunity to investigate the combinatorial impact of these variables in promoting not only dentin tissue regeneration and the development of new blood vessels, which is required for effective tissue regeneration.

DPSCS AND ANGIOGENESIS

The relationship between DPSCs and angiogenesis includes this specialised stem cell found within the dental pulp and the process of creating new blood vessels. Angiogenesis is a fundamental biological process that is crucial in tissue regeneration, wound healing, and various pathological conditions (Tahergorabi & Khazaei 2012). DPSCs, as a type of mesenchymal stem cell, have been shown to have a significant impact on angiogenesis due to their unique properties and abilities (Mattei et al. 2021).

DIFFERENTIATION OF DPSC TO ENDOTHELIAL CELLS

DPSCs have the potential to differentiate into various cell types, including endothelial cells, which are the building blocks of blood vessels (Yuan et al. 2022). When DPSCs are introduced into a microenvironment that requires tissue regeneration, they can respond to specific signals and cues, often released during injury or inflammation, and contribute to angiogenesis through several mechanisms (Ogata et al. 2022). The common signals that contribute to this process involve several factors including VEGF, and FGF (Allah et al. 2020; Gharaei et al. 2018). Within DPSCs, these growth factors activate signalling pathways that govern gene expression and cellular behaviour. The DPSCs begin to alter morphologically over time (Guerrero & McCarty 2017; Shibuya 2011). They change from a mesenchymal stem cell-like form to a more elongated, spindle-shaped morphology similar to endothelial cells (Ferro, Spelat & Baheney 2014; Staniowski, Zawadzka-Knefel & Skośkiewicz-Malinowska 2021). Moreover, in cell-to-cell interaction, DPSCs can interact directly with endothelial cells and other cells involved in blood vessel formation. These interactions can influence the behaviour of both DPSCs and endothelial cells, fostering a microenvironment conducive to angiogenesis (Sasaki et al. 2020). DPSCs' gene expression profile changes as the differentiation process advances. Endothelial cell marker genes such as CD31 (also known as PECAM-1), VE-cadherin, and von Willebrand factor are increased, suggesting the development of endothelial features (Mbagwu & Filgueira 2020). Once differentiation has been established, the newly created endothelial cells may be developed and grown in culture, supplying a source of functional endothelial cells for a variety of applications (Liu et al. 2018).

The differentiation of DPSCs into endothelial cells has promise for various therapeutic applications, including the treatment of vascular disorders, wound healing, and the generation of transplantable vascularised tissues. However, it is crucial to highlight that research in this area is continuing and optimising the differentiation techniques as well as assuring the functioning and safety of the resultant endothelial cells are currently active areas of interest in the field of regenerative medicine.

RELATIONSHIP OF DPSCS WITH ANGIOGENIC FACTORS

In paracrine signalling, DPSCs are known to secrete a range of bioactive molecules, including growth factors and cytokines, that are involved in the promoting angiogenesis. For example, DPSCs can release angiogenic growth factors such as VEGF, FGF2, and Ang-1. These secreted factors can attract and stimulate endothelial cells to migrate, proliferate, and form new blood vessels (Bar, Lis-Nawara & Piotr 2021). Moreover, in cell-to-cell interaction, DPSCs can interact directly with endothelial cells and other cells involved in blood vessel formation. These interactions can influence the behaviour of both DPSCs and endothelial cells, fostering a microenvironment conducive to angiogenesis (Bar, Lis-Nawara & Piotr 2021). In addition, this differentiation allows DPSCs to directly contribute to the process of angiogenesis (Saghiri et al. 2015). When DPSCs are stimulated with angiogenic factors, they display increased vasculogenic potential. This means they are more likely to develop into endothelial cells and contribute to blood vessel development (Bar, Lis-Nawara & Piotr 2021; Lamalice, Le Boeuf & Huot 2007). This characteristic is beneficial for vascularising tissues and promoting the regeneration of damaged blood vessels. When DPSCs are incorporated into a scaffold or matrix designed for tissue regeneration, they can contribute to the formation of vascular networks within the regenerating tissue (Mattei et al. 2021). Kwak and Lee (2022) study has shown that DPSCs can be primed or engineered to enhance their angiogenic potential. This can be achieved through genetic modifications or by exposing DPSCs to specific growth factors that amplify their pro-angiogenic properties. In general, DPSCs and angiogenesis are a dynamic interplay in which these stem cells contribute to the formation of new blood vessels, which, in turn, are vital for delivering nutrients, oxygen, and immune cells to regenerating tissues (Mattei et al. 2021).

The interaction between DPSCs and angiogenic factors continues to be used for medicinal applications. Researchers can modify DPSCs before transplantation by exposing them to particular angiogenic agents *in vitro* to stimulate their development into endothelial cells. This can help in vascular repair in ischemic disorders, wound healing, and tissue engineering. To summarise, DPSCs and angiogenic factors have a tight and dynamic relationship. They may both create and respond to these elements in their surroundings. This relationship makes DPSCs a

significant resource in regenerative medicine applications for stimulating angiogenesis and vascular repair, with the potential to develop therapies for diverse vascular disorders and tissue regeneration requirements. Studies have explicitly focused on using DPSCs to investigate the angiogenesis process. Although these studies underscore the inherent versatility of DPSCs, further comprehensive research is needed to unravel the finer intricacies of the DPSC-angiogenesis relationship. This evolving understanding could ultimately show the latent potential of DPSCs as a valuable asset in amplifying the creation of new blood vessels, thus improving the overall process of vascularisation in the context of tissue engineering and regenerative applications.

INTERPLAY BETWEEN ANGIOGENESIS AND DENTINOGENESIS

Several studies have documented increased angiogenesis when DPSCs are utilized, especially in conjunction with polymer scaffolds, as these nine papers demonstrated. Angiogenesis-related markers including CD31 and VEGF were consistently higher in various investigations. The angiogenic response was highly impacted by the type and composition of polymer scaffolds, with different synthetic and natural polymers exhibiting differing degrees of efficacy in stimulating blood vessel creation. The studies also showed that DPSCs might develop into cells resembling odontoblasts, which would aid in the creation of dentin. The expression of dentin-specific markers including DMP1 and DSPP provided evidence of this. In addition to maintaining the structural integrity, the polymer scaffolds seemed to promote the differentiation of DPSCs into odontoblasts, indicating a synergistic effect.

The interplay between angiogenesis and dentinogenesis is supported by several interrelated factors. First, enhanced vascularization is pivotal, as angiogenesis ensures that differentiating cells receive the necessary nutrients and oxygen. This improved blood supply not only supports cell survival but also creates an optimal microenvironment conducive to odontoblast differentiation and subsequent dentin formation. The presence of a robust vascular network is critical for the effective regeneration of dental tissues, as it enables the delivery of essential growth factors and oxygen to the developing tissues (Adair & Montani 2011). On the other hand, DPSCs secrete various growth factors that are crucial for both angiogenesis and dentinogenesis. For example, VEGF is instrumental in promoting the formation of new blood vessels, thereby enhancing vascularization. Additionally, VEGF influences the differentiation of DPSCs into odontoblast-like cells, which are essential for dentin formation (Divband et al. 2022). This dual role of growth factors underscores the interconnected nature of these processes, highlighting how the promotion of angiogenesis can simultaneously support dentinogenesis. Furthermore, the polymer scaffolds used in these studies play a significant role in mediating the interaction between DPSCs and their microenvironment. These scaffolds provide a physical support structure that helps DPSCs adhere, proliferate, and differentiate (Kenakin 2019). The specific properties of the scaffolds, such as porosity and biodegradability, are crucial in regulating the release and distribution of growth factors. Porosity affects nutrient and oxygen diffusion, while biodegradability ensures that the scaffold gradually supports tissue formation without obstructing the regenerative process (Zielińska et al. 2023). Together, these scaffold properties contribute to a well-regulated environment that supports both angiogenesis and dentinogenesis, enhancing the overall efficacy of regenerative strategies in dental tissue engineering.

CONCLUSIONS

In conclusion, research on the complex interaction between DPSCs, polymer scaffolds, and angiogenesis is limited. Despite DPSCs, scaffold materials, and angiogenic agents have all been the focus of individual studies, their combined impact on angiogenesis and tissue regeneration has not been well examined. Although DPSCs have the capacity to differentiate into a variety of cell lineages, further research is still needed to understand how they behave in response to scaffolds and angiogenic stimuli. Examining how DPSCs adjust to scaffold settings may provide information about blood vessel development and tissue regeneration. By integrating gene and protein expression studies with scaffold-based DPSC cultures, the intricate processes via which DPSCs support angiogenesis and tissue regeneration may be clarified. This unexplored area holds significant promise for tissue engineering and regenerative medicine applications, potentially transforming tissue revitalisation and regenerative therapies beyond dentistry. While this scoping review provides valuable insights into the angiogenic effects of dental pulp stem cells (DPSCs) cultured on polymer scaffolds, several limitations must be acknowledged. The review includes a limited number of studies, which may restrict the comprehensiveness and generalizability of the findings. Additionally, the lack of assessment of the methodological quality of the included studies and the potential risk of bias further undermine the reliability of the results. Furthermore, the exclusive focus on the angiogenic effect of DPSCs on polymer scaffolds means that other potentially relevant factors and contexts were not considered. Future research should address these limitations by incorporating a larger number of high-quality studies, assessing methodological rigor, and exploring a broader range of applications and effects of DPSCs.

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