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Review Article

Self-Assembling Peptide Nano fibrous Hydrogel Scaffold (Puramatrix™) in Regenerative Endodontics

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Abstract

Regenerative Endodontics is a novel discipline within Dentistry that aims to treat necrotized and infected teeth through regeneration, repair and restoration of the dentin-pulp complex. PuraMatrix™ is a commercially-available self-assembling peptide nano fibrous scaffold with appealing properties for dental pulp regeneration. This review provides an up-to-date overview of the prospective use of PuraMatrix™ for dentin-pulp regeneration.

A structured and systematic literature search and analysis was performed. PuraMatrix™ supports survival, proliferation and migration of hDPSC/hSHED without interference with odonto-/osteo-genic differentiation. Culture of stem cell/PuraMatrix™ constructs supplemented with induction media resulted in increased ALP activity and formation of calcium salt deposits. Same constructs in normal medium co-cultured with tooth slices or root segments showed increased expression of odontoblastic markers (DSPP, DMP-1, MEPE). In animal studies, hDPSC/PuraMatrix™ hydrogel constructs subcutaneously implanted in SCID mice produced highly-vascular mineralized tissues with strong osteogenic marker expression (Parathyroid Hormone receptor, Osteopontin, Osteocalcin, Osteonectin). Same constructs inserted into root segment implants formed vascularized pulp-like tissues which occupied the whole extension of the root canal and produced new dentin.

Although the number of studies identified can be considered a limitation, available/accruing evidence suggests that self-assembling peptide PuraMatrix™ is a promising nano fibrous hydrogel scaffold for stem cell-based engineering and regeneration of functional dental pulp tissues. Additional studies are deemed necessary to assess safety, efficacy, impact potential and cost-effectiveness of this new technology on the future of Regenerative Endodontics and beyond.

Keywords: *Dental Pulp; Hydrogel; Nano fiber; PuraMatrix; Regenerative Endodontics; Stem Cells; Tissue Engineering*

Abbreviations: MTA: Mineral Trioxide Aggregate; hSCAP: Human Apical Papillae Stem Cell; hDPSC: Human Dental Pulp Stem Cell; hSHED: Human Exfoliated Deciduous Teeth Stem Cell; HUVEC: Human Umbilical Vein Cord Cell; DSPP: Dentin Sialo-Phosphoprotein; DMP-1: Dentin Matrix Protein-1

Introduction

Caries, dental trauma and forceful orthodontic treatments which intrude or extrude teeth may result in dental pulp necrosis. In young teeth, necrosis interrupts root development leading to incomplete dentinogenesis, narrow dentin walls and large pulp chambers which

increase the risk of fracture upon trauma and reduce survival rate of the affected teeth [1]. While traditional endodontic treatment for such cases (usually by means of multiple visit apexification using calcium hydroxide and other materials) allows control of the infection with reasonable success rates in terms of periapical healing; it does not provide the necessary stimulus to regenerate the dentin-pulp complex, resume root development or strengthen root structure; reason for which long-term structural integrity of these teeth remains compromised [1,2].

Recent advances in stem cell biology, genetics and tissue engineering gave raise to "Regenerative Endodontics"; a novel field within dentistry which aims to treat (repair, restore and regenerate) necrotized and infected immature teeth via the regeneration of the dentin-pulp complex. The original concept dates back to 1932, when stomatologist G.L. Feldman first proposed the use dentine fillings in order to stimulate remaining viable dental pulp cells within root canals to proliferate and regenerate continuing interrupted root development [2]. Since then, numerous regenerative approaches have been developed and tested including root canal revascularization, postnatal stem cell therapy, scaffold implantation, pulp implantation, 3D cell printing and gene therapy [2]. It is noteworthy that Root canal revascularization is the only aforementioned method currently approved for routine clinical application. Treatment is based upon the principle that under ideal circumstances [i.e. tight coronal tooth seal, absence of bacteria and necrotic tissues within root canal system, presence of an adequate intra-canal blood-derived scaffold, etc …], viable progenitor cells from the surrounding peri-apical tissues may re-populate root canal space differentiating into primary odontoblasts which re-establish the dentin-pulp complex for resuming normal root development [1]. Retrospective outcome studies of this approach demonstrate that root canal revascularization successfully increases both, tooth length and dentin wall thickness; with treated teeth exhibiting significantly higher survival rates and radiographic healing (of periapical lesions) when compared to the previous calcium hydroxide apexification or MTA apical plug approaches/strategies. Nonetheless, similar studies have reported several un-favorable outcomes including: (a) higher risk of tooth discoloration (derived from the use of minocycline as a root canal antibiotic), (b)unpredictable root development in long-term pulp necrosis cases (> 6 months, presumably due to absence of viable periapical progenitor cells) and (c) empty root canal spaces (presumably due to lack of tissue regeneration) [1]. Additionally, accumulating evidence obtained from pre-clinical and animal/*in vivo* studies shows that root canal revascularization is not predictable in terms of which type of tissue is formed within the root canal space. Indeed, studies often report the presence of cementum-like, bone-like and periodontal ligament-like tissues instead of dentin-pulp complex structures [3-5]. Similar results (Table 1) have been reported in limited human case reports [6-8], suggesting that the foreseen biological outcomes of root canal revascularization are not predictable and may not allow for true dentin-pulp regeneration to happen [1,3-5].

	Re-vascularization	Stem Cell/PuraMatrix™
Application	Clinical	Research
Colonizing cells	Random progenitor cells from periapical tissues (probably hSCAP) [1].	Customized according needs (i.e. hDPSC, hSHED or HUVEC).
Dental-pulp regeneration	Unpredictable (formation of cementoid, osteoid and periodontal ligament-like tissues) (3–5)	Osteoid and Dentin pulp-like tissues [14-16].
Dentin formation	Yes [1].	Yes [16].
Root length	Increased [1].	Not reported.
Root wall thickness	Increased [1].	Increased [16].
Tooth survival	Increased [1].	Unknown.
Disadvantages	Risk of tooth discoloration (derived from antibiotic use) [1]. Unpredictable results in long-term pulp necrosis cases (> 6 months) [1]. Empty root canals [1].	Higher cost of implementation.

Table 1: *Re-vascularization vs. Stem Cell/PuraMatrix™ Dental Pulp Regeneration.*

Furthermore, the regeneration of cementoid, osteoid and periodontal ligament tissues within root canals treated with re-vascularization approaches may relate to the peri-apical origin of colonizing progenitor cells which are theorized to be "Apical Papillae Stem Cells" or SCAP. SCAP are highly un-differentiated/multi potent stem cells responsible for radicular pulp and root-complex formation. As such, they are capable to differentiate intodentin-pulp, cement, alveolar bone and periodontal ligament structures [9]. Absence of specific differentiation cues within the re-vascularized root canals may induce SCAP to indiscriminately differentiate into peri-apical tissues instead of dentin-pulp structures; resulting in failed endodontic regeneration. In this context, the use of alternative progenitor cell lines with preference for dentin-pulp structure formation may be a solution. Within the oral mesenchymal stem cells, Dental Pulp Mesenchymal Stem Cells (DPSC) and Exfoliated Deciduous Teeth Stem Cells (SHED) show this quality and potential. Both (Table 2) have been reported to contain specific sub-populations capable to differentiate into odontoblasts, neurons and endothelial cells [9], all crucial for the successful regeneration of functional dental pulp with appropriate immune responsiveness, vitality and sensibility [1]. However, one of the main challenges for the clinical application of DPSC and SHED continues to be the need for an appropriate scaffold or delivery system; which allows both, release and support of cells within root canals. From a clinical stand point, an ideal scaffold can be expected to: (a) Adapt and model root canal anatomy, (b) Setin a reasonable clinical time, and; (c) Orient DPSC and SHED towards odontoblast differentiation. Thus, the commercially-available peptide nano fibrous hydrogel scaffold known as PuraMatrix™ (BD Bioscience, Bedford, MA) seems to be a naturally-appealing solution and strategy for dental pulp regeneration (Figure 1). Briefly, the material is characterized by: (a) Liquid/solution and may be easily and directly injected into the pulp chamber and the root canals [10], (b) Self-polymerizes rapidly under physiological conditions, forming a solid 3-Dhydrogel which also encapsulates cells (allowing adequate delivery into target structures) and supports their survival and proliferation [11,12], (c) May be customized at the molecular level according to special or individual needs [13]. Hence, the purpose of this review is to provide an up-to-date overview on the use and regenerative potential of the PuraMatrix™ in Regenerative Endodontics.

	hDPSC	hSHED	hSCAP
Origin	Dental pulp of permanent teeth.	Dental pulp of temporal teeth.	Apical Papillae.
Most common method for isolation	Enzymatic digestion.	Enzymatic digestion.	Enzymatic digestion or explants culture.
Proliferation Velocity	Intermediate.	Fast.	Fast.
Superficial markers	(+): CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD146, STRO-1. (-): CD14, CD19, CD24, CD34, CD45 HLA-DR.	(+) Oct14, CD13, CD29, CD44, CD73, CD90, CD105, CD146, CD166. (-): CD14, CD34, CD45.	(+): CD13, CD24, CD29, CD44, CD73, CD90, CD105, CD106, CD146. (-): CD18, CD34, CD38, CD45, CD150.
Differentiation Potential *in vitro*	Odontoblasts, Osteoblasts, Condrocytes, Adipocytes, Miocytes, Neurons, Endothelial Cells, Corneal Epithelial Cells and Melanocytes.	Odontoblasts, Osteoblasts, Chondrocytes, Adipocytes, Neurons, endothelial cells.	Odontoblasts, Osteoblasts, Condrocytes, Adipocytes.
Dentin-Pulp Differentiation-Potential *in vivo*.	Formed functional dentin-like structures with dentin/pulp complexes.	Formed dentin-like tissues but fail regenerating dentin/pulp complexes.	Induced whole root formation.

Table 2: *hDPSC, hSHED and hSCAP characterization [9,18].*

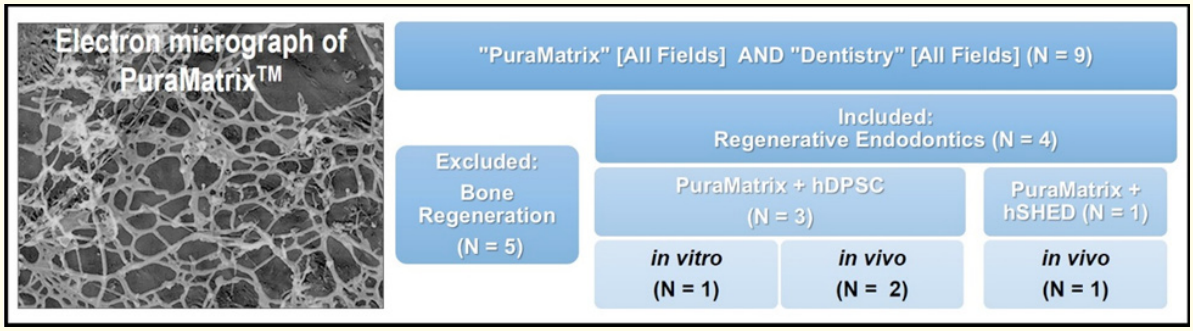

Figure 1: *Electron Micrograph of PuraMatrixTM Peptide Hydrogel and Flow Chart of followed Strategy/Results in this Systematic Literature Review.*

Materials and Methods

A structured literature search (Figure 1) was performed on PUBMED (May - July 2015) using the search terms "PuraMatrix" and "Dentistry" according to the following search strategy: "PuraMatrix [all fields] AND Dentistry [all fields]". Results were limited by: language (English) and full text availability. A total of 9 articles were found. Focus was centered on the use/application of self-assembling peptide hydrogel PuraMatrix™ in Regenerative Endodontics; reason why a total of 5 articles (related to bone regeneration) were excluded from this review. Data from the remaining studies was abstracted and compiled in tables and latter appraised by the authors. It is noteworthy that an additional manual search on PuraMatrix™'s official web-page (www.puramatrix.com) was performed to fetch for published or unpublished articles; however none in regenerative endodontic were found.

Results and Discussion

A total of 9 articles dealing with the potential use and application of PuraMatrix™ in Dentistry were found. Only 4 articles explored the prospective application of the material to regenerate the dental-pulp complex [10,14-16]. All studies were published recently (2011, 2013 and 2015) indicating that this particular topic and biomaterial is a novel field of research and development within contemporary Regenerative Endodontics. As a synthetic self-assembling peptide extracellular matrix, PuraMatrix™'s application requires combination with cells in order to promote tissue regeneration. Main cellular lines reviewed in the studies corresponded to: (a) Human Dental Pulp Stem Cells (hDPSC, 3/4 studies) [10,14,15], (b) Human Exfoliated Deciduous Teeth Stem Cells (hSHED, 1/4 studies) [16] and (c) Human Umbilical Cord Vein Cells (HUVEC, 1/4 studies) [14]. Most cell lines were tested in mono-cultures, except for hDPSC and HUVEC which were also co-cultured in one study [14]. Overall, the tested PuraMatrix™ concentrations ranged from 0.05% to 0.25%, (with 0.15% and 0.2% being the most recurrent for *in vivo* investigation).Regarding *in vitro* models, cell/biomaterial constructs were tested either alone (15,16) and/or combined with: (a) tooth slices (onto which constructs were seeded) [10] or (b) human root canals (into which constructs were injected) [14,16]. Pre-clinical testing models employed SCID mice and investigated the subcutaneous implantation of: (a) cell/biomaterial gel constructs [15] and (b) roots with injected cell/biomaterial constructs [14,16]. For the benefit of the reader, consult: Figure 1 and Table 3.

PuraMatrix™ supports *in vitro* survival, proliferation and migration of hDPSC and hSHED

hDPSC: Available *in vitro* evidence from mono-culture hDPSCs seeded in PuraMatrix™ show that cells survive and successfully proliferate in spite of the concentration of the biomaterial (which ranged from 0.05% to 0.25% in reviewed studies) [10,14]. Findings are as remarkable as previous studies with other primary cell lines reporting that variations in PuraMatrix™'s density often altered cell growth (i.e. 0.15% PuraMatrix™ is the optimal concentration for HUVEC cell viability compared to 0.5% and 0.25% concentrations) [10,14]. Initially (first 24 hours), encapsulated hDPSC appeared as "tiny round peas" evenly dispersed within the hydrogel [15]. At this stage, some cells seemed to develop primitive "cell processes" which oriented and extended towards nearby cell-clusters. After 3 days of culture, hDPSC within PuraMatrix™ resumed former "spindle-like" appearance while cell extensions appeared more mature into what

the authors described as "cytoplasmic elongations" [10]. By 2 weeks of culture, individual hDPSC could not be identified using contrast light microscopy. According to authors, this is mainly due to increased cell cluttering and extracellular matrix build-up resulting from extensive proliferation [15]. Using laser confocal images, the researchers reported that hDPSC no longer distributed homogenously within the PuraMatrix™. Instead, cells aligned and connected forming thread-like structures highly interwoven into PuraMatrix™'s 3D configuration [15]. By the fourth week of culture, hDPSC began spreading outside the biomaterial forming large colonies of adherent cells onto the culture-ware. Interestingly significant mineralized deposits nearby these colonies, which resulted to be calcium depositions according to Alizarin Red Staining, were reported [15]. Together, these observations support the potential application of PuraMatrix™ in dental pulp regeneration, where it seems that the biomaterial serves as a functional and cytocompatible nano fibrous hydrogel scaffold/carrier, without interfering with hDPSC growth.

hSHED: Mono-cultured hSHED in 0.2% PuraMatrix™ reported similar results to those of hDPSC. It was demonstrated that cells remained viable and actively proliferating, reaching a 4-fold increase in cell number after 7 culture days [16]. Seeded hSHED initially exhibited a round shape with cluster organization (24 hours), yet, as time progressed (7 days), the cells changed their phenotype into a characteristic spindle-like form [16].

REF	Design	PuraMatrix™	Cells	Main Outcomes
[10]	*in vitro*	0.05% – 0.25% co-cultured with tooth slices.	hDPSC (4^{th} to 8^{th} passage)	↑ Survival, proliferation and expression of odontoblastic markers (DSPP and DMP-1).
[16]	*in vivo* (subcutaneous implantation in SCID mice)	0.2% injected into root segments.	hSHED (3^{rd} to 8^{th} passage)	↑ Survival, proliferation and expression of odontoblastic markers (DSPP, DMP-1 and MEPE). *In vivo* formation of vascular-rich pulp-like tissues within root canals, dentin neo-formation.
[14]	*in vivo* (subcutaneous implant in SCID mice)	0.5%, 0.15%, 0.25% injected into root segments.	hDPSC +/- HUVEC (3^{rd} to 6^{th} passage)	↑ Survival, proliferation, VEGF production and differentiation / mineralization (measured by ALP activity and Von Kossa staining). *In vivo* formation of vascular-rich pulp-like tissues within root canals [Combination with HUVEC enhanced outcomes and promoted fast vessel network development (24 hours *in vitro*)].
[15]	*in vivo* (subcutaneous implant in SCID mice)	0.5 %	hDPSC (2^{nd} passage)	↑ Survival, proliferation, differentiation (measured by Alizarin Red staining). *In vivo* formation of highly vascularized / mineralized tissues, increased expression of osteogenic markers (Paratyroid hormone receptor, osteonectin, osteocalcin, osteopontin). No adverse or inflammatory reactions were reported.

Table 3: *PuraMatrix™ in Dental Pulp Regeneration.*

hSHED: Regarding odontoblastic differentiation of hSHED, limited evidence obtained from Rosa., *et al*. [16] where similar results to those of hDPSC were reported Re-suspended hSHED/0.2% PuraMatrix™ constructs injected into human root canals exhibited an increased expression of the odontoblastic markers DSPP, DMP-1 and MEPE after 21 days of culture [16]. PuraMatrix™ by itself (without injection into human roots) was not able to induce the expression of any of the aforementioned markers [16], suggesting, once again, that dentin-derived growth factors are vital or key for odontoblastic differentiation of oral mesenchymal stem cells.

PuraMatrix™ generates *in vivo* dentin-pulp-like structures

hDPSC: Chan., *et al*. [15] constructed hDPSC/0.5% PuraMatrix™ hydrogel for subcutaneous insertion into immuno-compromised nude mice. Complete transformation into solid chunks or pieces of viable tissue resulted after 4 weeks of implantation [15]. This contrasted with plain-gel PuraMatrix™ controls (no cells) which could not be found/located for retrieval; suggesting complete resorption of the biomaterial without new tissue formation. 2D-Radiografic analyses of the constructs revealed the presence of multiple mineralization foci which (according to light microscopy analysis) coincided with regions of nuclear sparse extracellular matrix of lobular appearance. Further, higher magnification revealed rich vascularization and distinct outline of each lobule; while gaps in-between lobules were filled with tissue of higher nuclei density [15]. While circumferential apposition of cells contouring each lobule was noticed, no inflammatory infiltrate or adverse reactions to the implant were reported. Further immunohistochemical analyses of same samples revealed the presence of large portions of in-between-lobule tissues stained positive for parathyroid hormone receptor (which is responsible for bone matrix formation), while osteopontin, osteocalcin and osteonectin expression was limited to the lobules themselves [15]. Aforementioned ostegenic markers were not equally expressed among the lobules; as follows: (a) Osteocalcin was the most widely distributed (mainly in large and medium-size lobules and occasionally in smaller ones), (b) Osteonectin was limited to the core of large lobules and (c) Osteopontin was found in smaller lobules [15]. Altogether, these results not only indicate that hDPSC/PuraMatrix™ gel constructs possess *in vivo* osteogenic capacity, yet also suggests that a specific pattern for lobule formation exists, characterized by: (a) initial formation of small osteopontin packed lobules, (b) progressive increasing in size with replacement of osteopontin for osteocalcin and (c) final replacement of osteocalcin for osteonectin and collagen type I in larger lobules [15]. A main critic for this study is the absence of a control hDPSC group without PuraMatrix™ to which one can compare the degree of differentiation/mineralization of treated tissues. As mentioned previously, *in vitro* evidence suggests that the role of PuraMatrix™ within odonto-/osteo-genic differentiation is supportive rather than inductive; hence it would have been interesting to evaluate pre-clinically/*in vivo* whether the incorporation of PuraMatrix™ clinically enhances differentiation. This is the subject of continuing trials within our BioMAT'X research group.

Dissanayaka., *et al*. [14] investigated hDPSC/PuraMatrix™ roots (main difference with previous hDPSC study which inserted the hydrogel freely) implanted subcutaneously in SCID mice. Vascularized pulp-like tissues resulted [14]. Antibody-staining against human mitochondria confirmed hDPSC as the main source of regenerated structures. On the contrary, PuraMatrix™ alone (without cells) root segments failed to produce tissues at all. Results indicate that PuraMatrix™ does not possess intrinsic ability to attract endogenous cells to populate the scaffold within root canals. An interesting observation was that the hDPSC/PuraMatrix™ constructs near the coronal aspect of the roots (which were sealed) did not survive, therefore dental pulp-like structures only formed up to the middle (5mm) or lower third (3.3mm) of the root canals [14]. Furthermore, in open apex teeth, host tissue in-growths tended to occur, pushing constructs further into the coronal aspects of the tested root canals [14]. From a clinical stand-point, both observations are interesting as: (a) immature open apex teeth are the primary target of Regenerative Endodontic procedures and (b) coronal root sealing is a requirement for endodontic treatment. Combined, this may challenge the clinical application of hDPSC/PuraMatrix™ constructs in Regenerative Endodontics; hence, is to be addressed in future investigations.

hSHED: Subcutaneous implantation of human premolar roots loaded with hSHED/0.2% PuraMatrix™ constructs in SCID mice resulted in the formation of new vascularized connective-like tissues, close to pre-dentin (whereas PuraMatrix™ alone formed minimal and poorly organized tissues).PuraMatrix™ engineered pulps occupied the whole extension of the root canal and had similar (a) cell proliferation (b) number of cells close to pre-dentin, (c) vessel density and (d) occurrence of cell apoptosis, to human dental pulp controls [16]. From a functional standpoint, tetracycline staining revealed that engineered structures generated new dentin throughout root

canal walls. Together with immunohistochemistry confirmation that newly formed tissues are primarily populated by hSHED [16]; PuraMatrix™ seems to be a proper delivery vehicle for hSHED in Regenerative Endodontics favoring cell differentiation into dental pulp-like structures within dental root canal.

Comments on the application of PuraMatrix™ in Regenerative Endodontics

Optimal Biomaterial Concentration: The concentration of PuraMatrix™ has no significant effect or influence on cell growth and proliferation [10,14]. Hence, the selection of the ideal concentration for endodontic applicationswill relies on other considerations. According to Calvancanti., *et al*. [10], the 0.2% concentration is optimal for regenerative endodontic applications as it increases the rigidity of the biomaterial resulting in a more suitable or appropriate gel for root canal injection and manipulation [10]. Similar observations were made by Dissanayaka., *et al*. [14], were they warned against using PuraMatrix™ in concentrations < 0.15%, as it increases fragility, rendering it difficult to handle and inject into the root canals.

Optimal model for *in vitro* and *in vivo* testing: As mentioned previously, present evidence suggests that the dentin-derived molecules are crucial for hDPSC and hSHED odontoblastic differentiation. Pre-clinical studies suggest that the residual dentin within tooth slices and root canals serve as a reservoir and source for these molecules, under experimental conditions [10]. Considering that root canal segments provide a much more similar environment than that of clinical endo-treated teeth (with sealed coronal aspect and narrow apical opening) [14]; this might be the ideal model for *in vitro* and *in vivo* investigation of PuraMatrix™/cell constructs. Also, root canal anatomy of segments allows testing the capacity of the engineered constructs to model the root anatomy in a reasonable clinical time; a crucial aspect for bench-top to chair-side translation of this technology.

hDPSC or hSHED mono-cultured vs. co-cultured with Human Umbilical Vein Cord Cells (HUVEC): Basic surgical and tissue engineering concepts state that vascularization is a key factor in tissue repair and regeneration. Rapid in vivo vascularization via intrinsic capillary formation of grafts and alternative bio-engineered implants (i.e. cell/PuraMatrix™ constructs) is critical for successful clinical outcomes. A major limiting/challenging factor in clinical application of endodontic regeneration procedures is the small diameter of the apical foramen, hindering the development and re-establishment of an adequate vascular supply during dental pulp regeneration. According to *in vivo* evidence discussed in this review, PuraMatrix™ scaffolds within root canals are poorly colonized by host cells; meaning that intrinsic vascularization depends almost exclusively on the seeded hDPSC or hSHED (which are capable to differentiate into endothelial cells). This phenomenon however, takes time and may be too slow for a successful clinical application. In order to promote and accelerate vascularization of hDPSC/PuraMatrix™ constructs, Dissanayaka and colleagues added commercial Human Umbilical Cord Vein Endothelial Cells (HUVEC, ScienCell Research Laboratories, San Diego, CA) to their preparations [14]. This unique approach proved to enhance (a) cell survival, (b) angiogenic factor production (VEGF), (c) vascular structure formation, (d) odonto-/osteo-genic differentiation and (e) extracellular matrix formation and collagen deposition, when compared to conventional hDPSC/PuraMatrix™ constructs. Excitingly, the vessels formed within the hDPSC/HUVEC/PuraMatrix™ constructs were 3D-organized and presented with larger lumen than that of the tubular structures within the HUVEC mono-cultures or HUVEC/hDPSC co-cultures. Moreover, the vascular structures appeared within only 24 hours of *in vitro* incubation, suggesting that it may be possible to pre-vascularize the PuraMatrix™/cell constructs before clinical implantation [14]. Despite such promising results, for a successful clinical application of this strategy, the following are to be considered:

a. Pre-clinical (*in vitro*) survival and proliferation of HUVEC in PuraMatrix™ depends on the biomaterial concentration. Using 0.15% PuraMatrix™ seems to be the "optimal" concentration for these cells.
b. HUVEC survived up to two weeks in mono-culture whereas co-cultured cells with hDPSC lasted longer. This indicates that HUVEC are extremely delicate and need the support from hDPSC in order to survive within the PuraMatrix™. Studies to identify the optimal hDPSC numbers for *in vitro* and *in vivo* support of HUVEC should be performed prior to clinical application.
c. Observed partial pulp regeneration (where dental pulp-like tissues formed up to first 3.3 mm-5 mm of the root canal and host-tissue invasion and coronal migration of the implant occurred in open apex teeth) indicates that both, critical size defect and critical apical opening size for pulp regeneration must be defined in order to assure a successful clinical application in the future [14].

Conclusions

PuraMatrix™ is a synthetic, injectable and self-assembling peptide nano fibrous hydrogel scaffold recently introduced into the emerging field of Regenerative Endodontics. The material is a promising scaffold alternative for dental pulp tissue engineering. PuraMatrix™ supports hDPSC and hSHED viability, proliferation and migration without interfering with cell odonto-/osteo-genic differentiation [suggesting that the main stimulus for differentiation originates from the hDPSC/hSHED themselves as well as the host tissues (especially dentin)]. Under *in vivo* experimental conditions, stem cell/PuraMatrix™ constructs seem to generate highly-vascularized dental pulp-like structures with mineralization potential. These engineered pulp-like structures contain functional odontoblasts capable to generate appositional dentin within root canal walls. Although the number of studies analyzed in this review is limiting, results suggest that the biomaterial might be of great potential when used of applied for stem-cell delivery and dental pulp regeneration within necrotized root canals. Future studies will explore the use, application and potential of PuraMatrix™ and further assess clinical safety, efficacy, significance, impact and cost-effectiveness of this biomaterial/strategy in dental pulp regeneration, and beyond.

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Conflict of Interest

Authors of this article declare having no conflict of interest.

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