 doi:10.20944/preprints202101.0398.v1

Article – Original research

Self-assembling peptide P_{11}-4 presents potential of inducing odontoblast-like cells to biomineralization and cell migration.

Isaac Jordão de Souza Araújo[1], Gustavo Narvaes Guimarães[2], Renato Assis Machado[3,4], Luiz Eduardo Bertassoni[5], Robert Philip Wynn Davies[6], Regina Maria Puppin-Rontani[7,*]

[1] Department of Restorative Dentistry, Dental Materials Area, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.; isaacjsouzaa@gmail.com
[2] Department of Biosciences, Histology area, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.; gu_narvaes@yahoo.com.br
[3]Hospital for Rehabilitation of Craniofacial Anomalies, University of São Paulo (HRAC/USP), Bauru, São Paulo, Brazil.
[4]Department of Oral Diagnosis, School of Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.; renatoassismachado@yahoo.com.br
[5]Department of Biomaterials and Biomechanics, School of Dentistry Center for Regenerative Medicine, Oregon Health and Science University, Portland, Oregon, United States.; bertasso@ohsu.edu
[6]Division of Oral Biology, School of Dentistry, University of Leeds, Leeds, United Kingdom.; R.P.W.Davies@leeds.ac.uk
[7]Department of Health Sciences and Pediatric Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.; rmpuppin@unicamp.br

* Correspondence: rmpuppin@unicamp.br

Abstract: Self-assembling peptide P_{11}-4 is amphiphilic and pH-triggered with demonstrated effectivity repairing early carious lesions in enamel. However, P_{11}-4 effects on dentin biomineralization and repair remain unexplored. Thus, cytocompatibility and effectiveness of P_{11}-4 inducing mineralization and migration of odontoblast-like cells (MDPC-23) were investigated. MDPC-23 were seeded in contact with P_{11}-4(0.5µg/ml and 1µg/ml), Dentin Matrix Protein 1 (DMP1 0.5µg/ml and 1 µg/ml) or Calcium hydroxide ($Ca(OH)_2$ 100µg/ml) solutions. Cytotoxicity was verified using MTT (n=6/group). Mineralization was tested using Alizarin Red (n=4/group). Cell migration was assessed by light microscopy (n=2/group). MTT and Alizarin Red data were compared using Kruskal-Wallis and Mann-Whitney (α=0.05). P_{11}-4 (0.5µg/ml and 1µg/ml) and DMP1 (0.5µg/ml and 1µg/ml) presented the highest cytocompatibility; $Ca(OH)_2$ presented the lowest. DMP1 1µg/ml exhibited the highest mineralization ability, with no difference to P_{11}-4 1µg/ml. $Ca(OH)_2$ presented lower values than DMP1 1µg/ml ($p<0.05$), but similar to P_{11}-4 1µg/ml. P_{11}-4 and DMP1 at 0.5 µg/ml showed induced less mineralization than P_{11}-4 and DMP1 at 1µg/ml ($p<0.05$), with no difference to $Ca(OH)_2$. All materials stimulated cell migration, however, lower concentrations of DMP1 and P_{11}-4 provided better results. P_{11}-4 is cytocompatible, induces mineralization and MDPC-23 migration like DMP1. P_{11}-4 could be an alternative for dentin mineralization and tooth repair.

Keywords: Self-assembling peptide; biomineralization; pulp-dentin complex; tooth repair.

1. Introduction

The response of the pulp-dentin complex to injuries is a natural defense mechanism to protect the dental organ from damage [1]. In order to maintain health and vitality, the pulp cells react against harmful stimuli synthesizing many proteins, which can mediate

 doi:10.20944/preprints202101.0398.v1

the formation of reactionary and reparative dentin [2] and participate in the biomineralization process [3].

Biomineralization involves the formation of complex, well-organized structures through a synergistic action between a protein matrix and minerals, where the matrix offers a template for the nucleation, growth, and organization of inorganic phases over multiple length scales [4]. Among these proteins, Dentin Matrix Protein 1 (DMP1) is an acidic matrix phosphoprotein that belongs to the Small Integrin-Binding N-linked Glycoproteins (SIBLING) family, which are associated with the formation of mineralized tissues [5]. DMP1 is related to the biomineralization process in dentin and can induce the differentiation of odontoblasts [6]. Furthermore, DMP1 is expressed in all odontogenic stages (dental lamina, enamel organ, and dental papilla) with some variation in the protein expression levels between each stage [7].

DMP1 plays an important role throughout the odontogenesis and dentin biomineralization, and it was recently evidenced that this protein remains adjacent to the pulp cavity, in peritubular zones, and it is less deposited near the enamel-dentin junction [8]. Complementary to that, DMP1 is also involved with the reparative process in dentin and contributes to the nucleation of amorphous calcium phosphate (ACP) and in the organization of apatite crystallites in intrafibrillar collagen zones [9, 10].

Despite the recent literature that has clarified the role of these small proteins in tooth repair and regeneration, most of the procedures concerning the protection of pulp-dentin complex, to induce reparative processes, are based on the use of calcium silicate and calcium hydroxide ($Ca(OH)_2$) materials [11]. The widespread use of $Ca(OH)_2$ is mostly related to its high alkalinity (pH ~12.5-12.8), allied to calcium and hydroxyl dissociation that induces mineral deposition and dentin bridge formation [12]. Moreover, $Ca(OH)_2$ also induces partial solubilization of dentin and the release of small amounts of bioactive molecules involved with the formation of dentin bridges [13]. However, $Ca(OH)_2$ generates an inflammatory response of the subjacent pulp tissue, leading to coagulative necrosis, after that, the mineralized barrier formation isolates and protects the pulp against initial injuries [14]. Apart from $Ca(OH)_2$ protective effects, the production of dentin bridges are not homogeneous and may act solely as a plug that tries to block damage progression.

In this sense, it is important to develop novel alternatives to induce dentin biomineralization, mimicking the processes that occur during odontogenesis, once the mineral deposition is not naturally dependent on inflammatory and necrotic processes [10]. For this purpose, self-assembling peptide P_{11}-4 is a promising candidate, which was inspired by the telopeptide at the C-terminal of the amelogenin. Amelogenin was previously thought to be involved only in enamel mineralization, but it has also been found to play an important role in the early stages of dentin formation, due to its transient expression by odontoblasts during pre-dentin deposition [15]. Therefore, it has been suggested that amelogenin may act as a signaling molecule during the initiation of hard matrix formation [15]. Moreover, it is proposed that amelogenin plays important roles not only on initial cytodifferentiation but also during tooth repair processes in humans since amelogenin is strongly re-expressed in newly differentiated odontoblasts and found to be distributed in the dentinal tubules under carious lesions in permanent teeth [16]. Thus, the use of a self-assembling peptide inspired by this protein could represent an interesting biomimetic alternative to repair and guide the biomineralization process in dentin.

P_{11}-4 in its simplest terms can be described as a monomeric peptide that in response to specific external triggers forms hierarchical structures due to the formation of β-sheets [17]. This peptide is effective in reducing demineralization and attracting ions to remineralize enamel subsurface lesions [18, 19]. Besides the promising results on enamel, de Sousa et al., 2019 [20] demonstrated that P_{11}-4 peptide is also capable of binding to type I collagen. Their findings indicated that P_{11}-4 increases the collagen fibrils' thickness,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 January 2021 doi:10.20944/preprints202101.0398.v1

which improves the resistance against the proteolytic activity. Furthermore, P_{11}-4 has negatively charged domains mimicking those found in the native macromolecules involved in the mineralization process. The negatively charged domains enhance its organization and it can be viewed as analog protein [18, 21]. Moreover, because of its mineralization potential, *de novo* calcium and phosphate nucleation were also analyzed, and the crystal diameter and microstructure presented a significant modification in the presence of P_{11}-4 [20].

Due to the inherent interaction of P_{11}-4 with type I collagen, its similarities to the macromolecules involved in the mineralization process, and calcium-phosphate nucleation, it is necessary to compare the effects of this peptide with compounds capable of inducing dental tissue mineralization to demonstrate its efficacy. One under-reported area is P11-4s' interaction with specific cell types. Therefore, this study focuses on the interaction of the self-assembling peptide with odontoblast-like cells. Here we assess the cytocompatibility, the mineral deposition, and potential of inducing cell migration, comparing its performance to $Ca(OH)_2$ and to DMP1, a well-known bioactive molecule involved in biomineralization. Based on the current literature we hypothesize that the self-assembling peptide is cytocompatible and induces mineralization, therefore it is potentially an appropriate material as an alternative therapy to replace calcium hydroxide in tooth repair and dentin regeneration as a pulp capping agent.

2. Materials and Methods

Cell culture

Immortalized odontoblast-like cell MDPC-23 were cultured in Petri dishes with DMEM (GIBCO, Auckland, NY, USA) medium, supplemented with 10% FBS (GIBCO) and 1% penicillin/streptomycin antibiotic (GIBCO) and incubated at 37 °C and 5% CO_2 until the pre-confluent stage was reached. Then, the cells were detached, counted, seeded (at a density of $7.5x10^4$ cells/ml) into cell culture plates and kept at 37 °C, 5% CO_2, before use. To compare the effects of the different biomineralization agents, the cells were cultured in the presence of $Ca(OH)_2$ (Sigma Aldrich, St. Louis, MO, USA) (100µg/ml), recombinant DMP1 (R&D Systems, Inc. Minneapolis, MN, USA) (0.5µg/ml and 1.0µg/ml) and, P_{11}-4 peptide (Curodont™ Repair, Credentis AG, Windisch, SWI) (0.5µg/ml and 1.0µg/ml). Also, a negative control, with no specific treatment was included in each assay.

Cell viability assay (MTT)

The cell viability was verified using 3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT). The cells were cultured into 96-wells plates, treated concerning the material and concentration (n=7), and incubated (37 °C and CO_2 5%) at 24h and 72h. After each period of incubation, the medium was aspirated, and the cells were washed with sterile phosphate buffer solution (PBS). Then, 150 µl of MTT solution was added to each well and incubated at 37 °C and 5% CO_2 for 4h. Subsequently, the MTT solution was aspirated and the formazan crystals precipitated were diluted with 150 µl of isopropyl alcohol. After the complete dissolution of formazan crystals, the plates were carried to a microplate reader (Biochrom Asys UMV340, Biochrom Ltd. Cambridge, UK). The absorbance was measured at 570 nm and the values of cell viability were expressed as a percentage.

Mineral deposition (Alizarin Red S)

The mineral deposition was evaluated using Alizarin Red S. The cells were cultured in an osteogenic medium with 2 mM β-glycerophosphate, 50 µg/ml ascorbic acid, and 1 µl/ml

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 January 2021 doi:10.20944/preprints202101.0398.v1

dexamethasone in a 24-well cell culture plate. A group with only osteogenic medium and a separate group containing only DMEM supplemented with 10% FBS was used as the positive and negative controls, respectively. The test groups were calcium hydroxide, DMP1, and P_{11}-4 at the same concentrations previously mentioned (n=4). The medium was changed every 3 days and the mineral deposition was evaluated after 21 days of culture. Alizarin Red S staining protocol was followed to measure mineralization [22, 23]. Briefly, after the culture period, the cells were washed with PBS and fixed with 4% paraformaldehyde for 1h at room temperature. The monolayer was washed twice with PBS, and then 1 ml of 40 mM Alizarin Red S solution (pH=4.1) was added to each well, left at room temperature for 20 minutes under gentle shaking. After, the solution was aspirated, and the wells were washed with distilled water 4 times to remove the unincorporated dye. Then, the wells were photographed with a camera (Nikon 3200, Nikon, Tokyo, Japan) to make the qualitative analysis.

To quantify the staining, 800 µl of 10% acetic acid was dispensed into each well and left for 30 minutes at room temperature with shaking. Next, the monolayers poorly attached to the well were aspirated and transferred to 1.5 ml microtubes and vortexed for 30 seconds. Then, 500 µl was added to the slurry, heated to 85 °C for 10 minutes, and kept in ice for 5 minutes and the slurry was centrifuged at 20,000 g for 15 min. After centrifugation, 500 µl of supernatant was transferred to a new 1.5 ml microtube. Then, 200 µl of 10% ammonium hydroxide was added to neutralize acetic acid. The absorbance was read in duplicate using 150 µl of the supernatant in a 96-well plate at 405 nm in a microplate reader.

Cell migration assay

The cells were cultured in 24-well plates and stored at 37 °C and 5% CO_2 until they reached the confluent stage. After confluence, a 1000 µl pipette tip was used to make a scratch in the cell monolayer. The cells were washed with PBS to remove debris and cells not attached to the plate. After debris removal, 500 µl of culture medium were added to each well, containing concentrations of each material, according to the groups previously described, including a control group with no treatment. Then, the plate was put into a 37 °C and 5% CO_2 chamber positioned in a 4X magnification at light microscope (Carl Zeiss, Oberkochen GER) adjusted to capture images of the wells for 72h with a digital camera (Axio Cam MRc, Carl Zeiss). Each group was tested in duplicate and the images (baseline and 72h) were visually analyzed to compare cell migration behavior regarding the treatments.

Statistical analysis

All data obtained from MTT and Alizarin Red S assays were submitted to the Shapiro-Wilk test to evaluate data distribution. Non-normal distribution was evidenced in both experiments and the data were analyzed by Kruskal-Wallis test and Mann-Whitney to compare groups (α=0.05), using the statistical software SPSS, version 21 (IBM statistics, Akron, NY, USA).

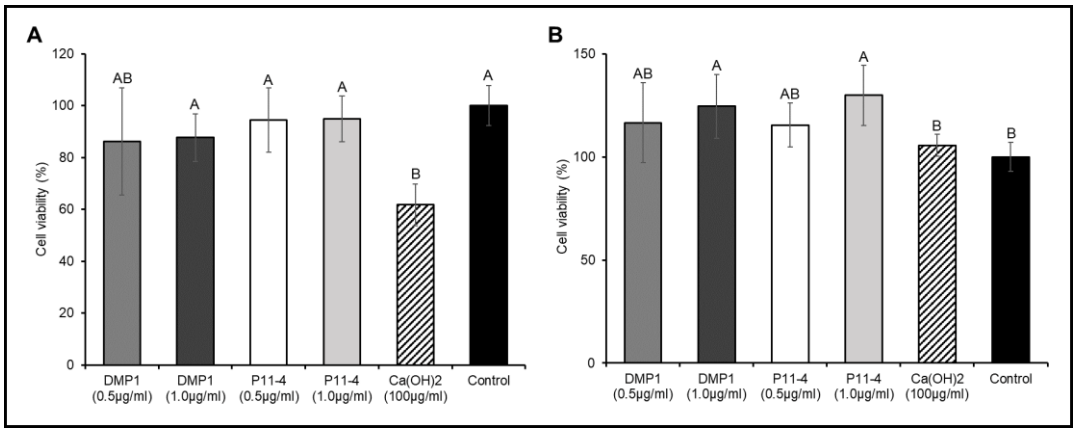
3. Results

3.1 MTT assay

The cell viability percentage for groups tested for 24 h and 72 h are shown in Figure 1. At 24 h, $Ca(OH)_2$ exhibited significant cytotoxicity. Meanwhile, the concentrations of DMP1 and P_{11}-4, appeared not to affect the cells, indicating more than 80% cell viability. After 72

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 January 2021 doi:10.20944/preprints202101.0398.v1

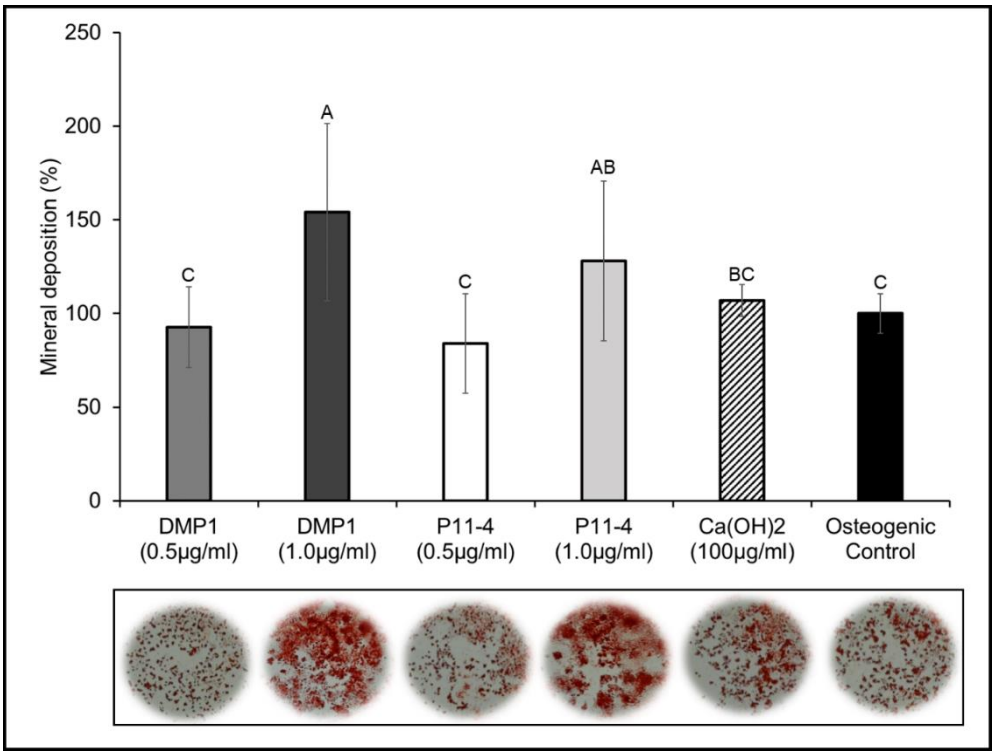
h, $Ca(OH)_2$ presented similar values compared to the control group (untreated cells). On the other hand, P_{11}-4 and DMP1 at 1 μg/ml exhibited significantly higher cell viability rather than $Ca(OH)_2$ and even the control. P_{11}-4 and DMP1 at 0.5 μg/ml presented slightly higher cell viability than $Ca(OH)_2$ but with no statistical significance.



ean and standard deviation of MDPC-23 cell viability (%) after 24h (A) and 72h (B). Different capital letters represent a statistical difference ($p<0.05$) between groups according to each time point. DMP1 and P11-4 were not cytotoxic. $Ca(OH)_2$ resulted in a significant drop in the cell viability at 24h ($p<0.05$). After 72h $Ca(OH)_2$ was similar to the control group, while DMP1 and P11-4 presented the highest cell viability values ($p<0.05$).

3.2 *Mineral deposition*

After 21 days of culture in osteogenic media, DMP1 (1μg/ml) exhibited the highest mineralization potential, while P_{11}-4 at the same concentration had demonstrated similar behavior. Although $Ca(OH)_2$ had lower mineralization values compared to DMP1 and P_{11}-4 (1μg/ml), it was higher than P_{11}-4 and DMP1 (0.5μg/ml). P_{11}-4 and DMP1 (0.5μg/ml) presented values even lower than the osteogenic control; however, no difference was found (Figure 2).



 doi:10.20944/preprints202101.0398.v1

Figure 2. Mean, standard deviation (top), and representative images (bottom) of mineral deposition after 21 days of culture in osteogenic media. Different capital letters represent statistical differences ($p<0.05$). DMP1 at 1.0 μg/ml presented the highest mineralization potential ($p<0.05$), which was not significantly different from P11-4 1.0 μg/ml. P11-4 1.0 μg/ml induced more mineral deposition than the control group and DMP1 and P11-4 at 0.5 μg/ml ($p<0.05$), but with no difference to $Ca(OH)_2$. DMP1 and P11-4 at 0.5 μg/ml had the lowest mineralization potential.

3.3 Cell Migration

All the experimental groups exhibited some level of cell migration directed towards the gap resulting from the removal of the cells. There was less visible migration of the control group compared to all different treatments. No completion in closing the wound was evidenced even with a remarkable migration exhibited by DMP1 0.5 μg/ml (Figure 3).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 January 2021 doi:10.20944/preprints202101.0398.v1

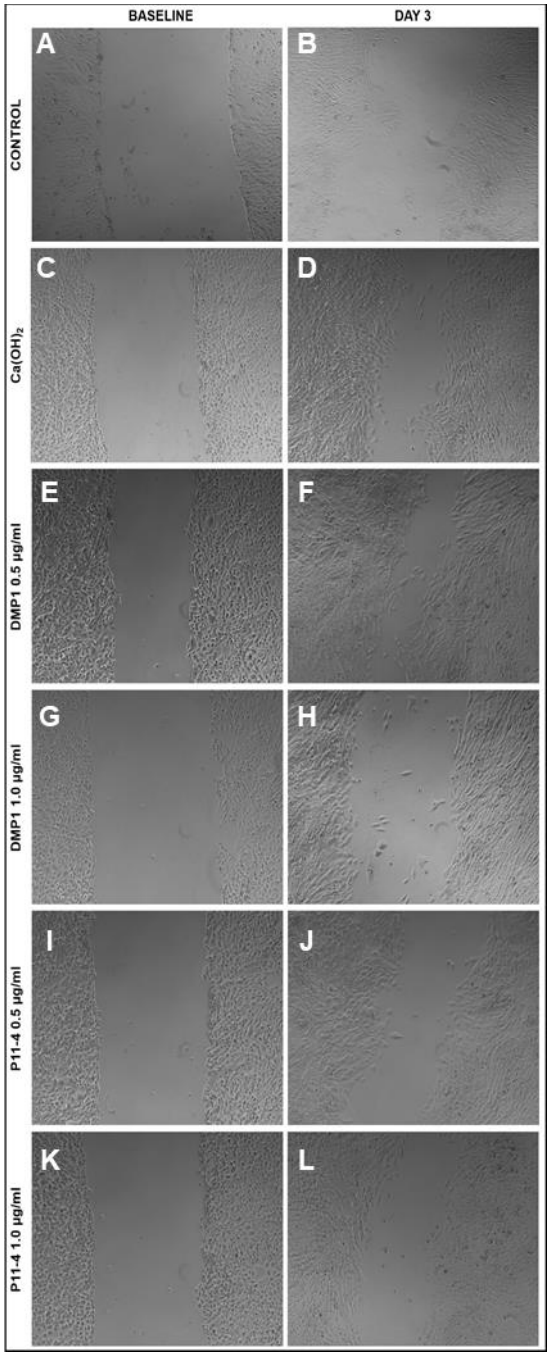


Figure 3. Representative SEM images for wound and healing experiments at baseline and after 3 days of migration. Control, with no treatment, (A-B); $Ca(OH)_2$ (C-D); DMP1 0.5 µg/ml (E-F); DMP1 1 µg/ml (G-H); P_{11}-4 0.5 µg/ml (I-J); P_{11}-4 1 µg/ml (K-L).

4. Discussion

Regenerative endodontic therapies have been widely discussed in the last years regarding pulp disinfection [24, 25], revascularization [26], and immune response [27] through different materials. However, the biomimetic approach is still an emerging alternative in pulp-capping procedures for deep cavities or pulp micro exposures.

The self-assembling peptide, P_{11}-4, effects on odontoblast-like cells were evaluated in this study, and the material presented cytocompatible characteristics. The cell response is a determinant factor for regenerative therapies [24, 28] and, in contrast to calcium hydroxide, which exhibited low cell viability at 24h but then recovered significantly after 72

 doi:10.20944/preprints202101.0398.v1

hours, P_{11}-4 demonstrated high biocompatibility at all time points, which suggests a non-inflammatory response and proliferative stimuli. Regarding cell migration and proliferation experiments, all the materials supported cell migration. However, the lowest concentration of DMP1 (0.5μg/ml) induced faster cell migration compared to other groups (Figure 3E-F).

P_{11}-4 at the highest concentration (1.0μg/ml) was effective in inducing mineral deposition in a similar way to DMP, both of which indicated superior results compared to calcium hydroxide. This inductive response and the cytotoxicity data bring insights to support the hypothesis that the self-assembling peptide could be used as a cell-friendly substitute for calcium hydroxide in the future. However, from the best of our knowledge, this is the first study using P_{11}-4 to stimulate odontoblast-like cells in dentin regeneration, then more studies to compare other parameters regarding the properties of the materials and the *in vivo* response are necessary.

Apart from that, the similarities in the results for P_{11}-4 and DMP1 could be associated with the negatively charged domains of the peptide amino acids sequence as a consequence of its three glutamic acids [29]. In addition, these findings could also be associated with the fact that the amino acid sequence of P_{11}-4 is similar to the residues of the amelogenin C-terminal, which plays an important role in the mineralization activity of that protein [30]. Carboxyl domains in peptides and proteins contribute to crystal nucleation by their repetitive organization, which can direct mineral arrangement [31]. On the other hand, the N-terminal helps in the stabilization of the amorphous mineral phase is important during crystal growth [32].

In the same way, DMP1 also has negatively charged domains and C- and N-terminals, which are, in fact, associated with the role of that protein on biomineralization [9,33]. Thus, the similarities effects of both peptide and protein were evidenced in this study and could be investigated in further studies. Moreover, the entire mechanism of the peptide, including its morphology and the role of biomineralization in this concentration regime should be clarified. However, it is known that the negatively charged domains, on the self-assembled surface, act as a heterogeneous nucleating template, dehydrating the calcium ion, producing nucleating centers [34].

P_{11}-4 can bind type I collagen to form thicker fibers, reduce proteolysis, and induce mineral nucleation in other *in vitro* models [20]. In addition, P_{11}-4 presented high levels of β-sheet formation [29] and had demonstrated a pH-responsive state transition that contributes to the kind of structure to form [17]. Besides, self-assembling peptides have stimulated mineralization biomarkers like alkaline phosphatase and osteocalcin in pre-osteoblast cells (MC3T3) [35] but, for P_{11}-4, no previous evidence of a modulatory effect for the odontoblast-like cell has been described. Thus, the possibility that the self-assembling peptide may behave as a synthetic analogous protein and plays a role in the biomineralization process, guiding the mineralization of the pre-dentin matrix, prospects future investigations in pulp-capping procedures and dentin regeneration strategies.

Although this is an *in vitro* study and P_{11}-4 was tested as a solution, which is hard to keep acting in a clinical situation, our data bring initial evidence to purpose P_{11}-4 as a synthetic biomimetic alternative in tertiary dentin formation. To confirm that, further studies are necessary to measure the effects of P_{11}-4 on molecular regulatory response. Besides, we are looking for carrying mechanisms to keep the peptide in contact with the damaged dentin or micro pulp exposures while it induces gradual biomineralization.

5. Conclusions

P_{11}-4 a self-assembling peptide showed no cytotoxicity effects, induced cell migration, and mineral deposition in odontoblast-like cells after 21 days. P_{11}-4 exhibited similar

values in all experiments compared to DMP1 at 1μg/ml and induced more mineral deposition than calcium hydroxide.

Author Contributions: Conceptualization, methodology, formal analysis, and validation I.J.S.A. and R.M.P-R.; investigation, I.J.S.A, G.N.G, and R.A.M; resources, R.M.P-R; data curation, I.J.S.A. and R.M.P-R.; writing—original draft preparation, I.J.S.A; writing—review and editing, I.J.S.A., G.N.G, R.A.M., L.E.B., R.P.W.D, and R.M.P-R.; visualization, supervision and project administration R.M.P-R.. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Coordination of Superior Level Staff Improvement (CAPES) Grant number: AUX/CAPES/PROEX:0878/2018.

Acknowledgments: The authors thank Dr. Marcelo Rocha Marques from the Histology Laboratory of Piracicaba Dental School for the collaboration.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Smith AJ, Tobias RS, Murray PE (2001) Transdentinal stimulation of reactionary dentinogenesis in ferrets by dentine matrix components. J Dent 29:341–346. https://doi.org/10.1016/S0300-5712(01)00020-3
2. Lee YL, Liu J, Clarkson BH, et al (2006) Dentin-pulp complex responses to carious lesions. Caries Res 40:256–264. https://doi.org/10.1159/000092235
3. Qin C, D'Souza R, Feng JQ (2007) Dentin matrix protein 1 (DMP1): new and important roles for biomineralization and phosphate homeostasis. J Dent Res 86:1134–1141. https://doi.org/10.1177/154405910708601202
4. Sprio S, Sandri M, Panseri S, et al (2014) Bone substitutes based on biomineralization. In: Bone Substitute Biomaterials. pp 3–29
5. Fisher LW, Fedarko NS (2003) Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. Connect Tissue Res. https://doi.org/10.1080/713713644
6. Almushayt A, Narayanan K, Zaki AE, George A (2006) Dentin matrix protein 1 induces cytodifferentiation of dental pulp stem cells into odontoblasts. Gene Ther 13:611–620. https://doi.org/10.1038/sj.gt.3302687
7. Martinez EF, Da Silva LAH, Furuse C, et al (2009) Dentin matrix protein 1 (DMP1) expression in developing human teeth. Braz Dent J 20:365–369
8. Beniash E, Deshpande AS, Fang PA, et al (2011) Possible role of DMP1 in dentin mineralization. J Struct Biol 174:100–106. https://doi.org/10.1016/j.jsb.2010.11.013
9. Deshpande AS, Fang PA, Zhang X, et al (2011) Primary structure and phosphorylation of dentin matrix protein 1 (DMP1) and dentin phosphophoryn (DPP) uniquely determine their role in biomineralization. Biomacromolecules 12:2933–2945. https://doi.org/10.1021/bm2005214
10. Niu LN, Zhang W, Pashley DH, et al (2014) Biomimetic remineralization of dentin. Dent Mater 30:77–96. https://doi.org/10.1016/j.dental.2013.07.013
11. Li Z, Cao L, Fan M, Xu Q (2015) Direct Pulp Capping with Calcium Hydroxide or Mineral Trioxide Aggregate: A Meta-analysis. J Endod 41:1412–1417. https://doi.org/10.1016/j.joen.2015.04.012
12. Rehman K, Saunders WP, Foye RH, Sharkey SW (1996) Calcium ion diffusion from calcium hydroxide-containing materials in endodontically-treated teeth: an in vitro study. Int Endod J 29:271–279. https://doi.org/10.1111/j.1365-2591.1996.tb01381.x
13. Graham L, Cooper PR, Cassidy N, et al (2006) The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components. Biomaterials 27:2865–2873. https://doi.org/10.1016/j.biomaterials.2005.12.020
14. Farhad A, Mohammadi Z (2005) Calcium hydroxide: a review. Int Dent J 55:293–301. https://doi.org/10.1111/j.1875-595X.2005.tb00326.x
15. Papagerakis P, MacDougall M, Hotton D, et al (2003) Expression of amelogenin in odontoblasts. Bone. https://doi.org/10.1016/S8756-3282(02)00978-X
16. Mitsiadis TA, Filatova A, Papaccio G, et al (2014) Distribution of the amelogenin protein in developing, injured and carious human teeth. Front Physiol 5:1–8. https://doi.org/10.3389/fphys.2014.00477
17. Aggeli A, Bell M, Carrick LM, et al (2003) pH as a trigger of peptide β-sheet self-assembly and reversible switching between nematic and isotropic phases. J Am Chem Soc 125:9619–9628. https://doi.org/10.1021/ja021047i
18. Kirkham J, Firth A, Vernals D, et al (2007) Self-assembling Peptide Scaffolds Promote Enamel Remineralization. J Dent Res 86:426–430. https://doi.org/10.1177/154405910708600507
19. Silvertown JD, Wong BPY, Sivagurunathan KS, et al (2017) Remineralization of natural early caries lesions in vitro by P 11 -4 monitored with photothermal radiometry and luminescence. J Investig Clin Dent e12257. https://doi.org/10.1111/jicd.12257

20. de Sousa JP, Carvalho RG, Barbosa-Martins LF, et al (2019) The Self-Assembling Peptide P 11 -4 Prevents Collagen Proteolysis in Dentin. J Dent Res 98:347–354. https://doi.org/10.1177/0022034518817351
21. Kind L, Stevanovic S, Wuttig S, et al (2017) Biomimetic Remineralization of Carious Lesions by Self-Assembling Peptide. J Dent Res 22034517698419. https://doi.org/10.1177/0022034517698419
22. Gregory CA, Gunn WG, Peister A, Prockop DJ (2004) An Alizarin red-based assay of mineralization by adherent cells in culture: Comparison with cetylpyridinium chloride extraction. Anal Biochem 329:77–84. https://doi.org/10.1016/j.ab.2004.02.002
23. Guimarães GN, Stipp RN, Rodrigues TL, et al (2013) Evaluation of the effects of transient or continuous PTH administration to odontoblast-like cells. Arch Oral Biol 58:638–645. https://doi.org/10.1016/j.archoralbio.2012.11.011
24. Albuquerque MTP, Valera MC, Nakashima M, et al (2014) Tissue-engineering-based strategies for regenerative endodontics. J Dent Res 93:1222–31. https://doi.org/10.1177/0022034514549809
25. Bottino MC, Pankajakshan D, Nör JE (2017) Advanced Scaffolds for Dental Pulp and Periodontal Regeneration. Dent Clin North Am 61:689–711. https://doi.org/10.1016/j.cden.2017.06.009
26. Athirasala A, Lins F, Tahayeri A, et al (2017) A Novel Strategy to Engineer Pre-Vascularized Full-Length Dental Pulp-like Tissue Constructs. Sci Rep 7:1–11. https://doi.org/10.1038/s41598-017-02532-3
27. Jeanneau C, Lundy FT, El Karim IA, About I (2017) Potential Therapeutic Strategy of Targeting Pulp Fibroblasts in Dentin-Pulp Regeneration. J Endod 43:S17–S24. https://doi.org/10.1016/j.joen.2017.06.007
28. Diogenes A, Henry MA, Teixeira FB, Hargreaves KM (2013) An update on clinical regenerative endodontics. Endod Top. https://doi.org/10.1111/etp.12040
29. Barco A, Ingham E, Fisher J, et al (2018) On the design and efficacy assessment of self-assembling peptide-based hydrogel-glycosaminoglycan mixtures for potential repair of early stage cartilage degeneration. J Pept Sci 24:. https://doi.org/10.1002/psc.3114
30. Ruan Q, Moradian-Oldak J (2015) Amelogenin and enamel biomimetics. J Mater Chem B 3:3112–3129. https://doi.org/10.1039/c5tb00163c
31. Yao S, Jin B, Liu Z, et al (2017) Biomineralization: From Material Tactics to Biological Strategy. Adv Mater 29:. https://doi.org/10.1002/adma.201605903
32. Gajjeraman S, Narayanan K, Hao J, et al (2007) Matrix macromolecules in hard tissues control the nucleation and hierarchical assembly of hydroxyapatite. J Biol Chem 282:1193–1204. https://doi.org/10.1074/jbc.M604732200
33. Oya K, Ishida K, Nishida T, et al (2017) Immunohistochemical analysis of dentin matrix protein 1 (Dmp1) phosphorylation by Fam20C in bone: Implications for the induction of biomineralization. Histochem Cell Biol 147:341–351. https://doi.org/10.1007/s00418-016-1490-z
34. Sam Alan Whitworth (2018) Self-Assembling Peptide Nano-Apatite Hybrid Material for Dentine Mineralisation. The University of Leeds
35. Horii A, Wang X, Gelain F, Zhang S (2007) Biological designer self-assembling peptide nanofiber scaffolds significantly enhance osteoblast proliferation, differentiation and 3-D migration. PLoS One 2:1–9. https://doi.org/10.1371/journal.pone.0000190