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Hydrogel Arrays and Choroidal Neovascularization Models for Evaluation of Angiogenic Activity of Vital Pulp Therapy Biomaterials

Mohammad Ali Saghiri^{1,2,*}, Armen Asatourian³, Eric H. Nguyen^{1,4}, Shoujian Wang¹, and Nader Sheibani^{1,2,4}

¹Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

²Department of Biomedical Engineering, University of Wisconsin – Madison, WI, USA

³Sector of Angiogenesis Regenerative Medicine, Dr.Hajar Afsar Lajevardi Research Cluster, Shiraz, Iran

⁴Human Models for Analysis of Pathways (Human MAPs) Center, University of Wisconsin, Madison, WI, USA

Abstract

Introduction: This study intended to evaluate the angiogenic properties of vital pulp therapy materials including white mineral trioxide aggregate (WMTA), calcium hydroxide (Ca(OH)₂), Geristore, and Nano WMTA biomaterials.

Methods: WMTA, Ca(OH)₂, Geristore, Nano WMTA disks were prepared, dispersed into 2 mL of MiliQ distilled water, and centrifuged to obtain 2 mL supernatant elution. Thirty five wells of poly ethylene glycol (PEG) hydrogel arrays were prepared and divided into five groups of seven (n=7). Mice molar endothelial cells (EC) were placed on hydrogel arrays. The elution prepared from each sample was diluted in growth medium (1:3) and was added to the hydrogel arrays. The EC medium alone was used for control. For choroidal neovascularization (CNV) model, thirty five 6-week-old female mice were lasered, divided into five groups, and elution from each sample (2 µl) or saline (control) was delivered by intravitreal injection on day of laser and one week later. The mean number of nodes and total branches length in hydrogel arrays, and mean area of CNV, were calculated using ImageJ software and analyzed by one-way ANOVA and Post hoc Tukey HSD tests.

Results: The comparison of results of number of nodes showed the values of control > Geristore > Nano WMTA > WMTA > Ca(OH)₂. In total branch length and CNV area, the comparison of results showed values of Geristore > control > Nano WMTA > WMTA > Ca(OH)₂.

*Corresponding Author: Mohammad Ali Saghiri D.Eng., MS, PhD, University of Wisconsin School of Medicine and Public health, Madison, WI 53705 USA, Saghiri@wisc.edu.

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Conclusions: All tested materials showed minimal antiangiogenic activity, while Geristore and Nano WMTA showed higher proangiogenic activity than WMTA and $\text{Ca}(\text{OH})_2$.

Keywords

$\text{Ca}(\text{OH})_2$; Geristore; Nano WMTA; WMTA; Tubulogenesis; Endothelial cells

Introduction:

Pulpotomy is defined as the surgical removal of inflamed coronal part of the dental pulp in order to protect and save the remaining healthy dental pulp tissue (1). The success rate of vital pulp therapy is strictly related to maintaining pulp vitality and homeostatic functions by restoration of the integrity of vascular network through angiogenesis. This is a determinant component that guides the regenerative procedures towards the survival or necrosis of pulp tissue.

Human dental pulp is a highly vascularized tissue, which due to its vascular network and progenitor or postnatal dental pulp stem cells (DPSC) have a great naturally inherent regenerative capacity (2). Although this issue might seem very simple in theory, the success in this procedure is closely related to the reestablishment of the homeostasis of dental pulp (3). Thus, maintaining the dental pulp blood supply, through the up- or down regulation of pro- or anti-angiogenic growth factors, is a key determinant component for regeneration of dental pulp tissue (4). In a recently reported study, the authors showed that application of pro-angiogenic agents such as “illoprost” during vital pulp therapy procedures can induce angiogenesis and formation of tertiary dentin. These authors demonstrated that the promotion in expression of angiogenic factors can enhance tertiary dentin formation in-vivo (5).

Many inorganic elements are recognized as being essential for tissue regeneration (6–8). $\text{Ca}(\text{OH})_2$ is able to release Ca ions and promote the migration of DPSC, enhance the production of biomolecules such as bone morphogenic proteins (BMP) and fibronectin (9). White mineral trioxide aggregate (WMTA), due to several advantages over $\text{Ca}(\text{OH})_2$, has become the most commonly used dental material in vital pulp therapy procedures (10). Geristore is a resin modified glass ionomer (RMGI) with composition of resin-based fluoro alumina silica glass (11). It was previously reported that glass ionomer cements might have inducing effect on proliferative activity in dental pulp such as $\text{Ca}(\text{OH})_2$ (1). Nano WMTA is a nano-modification of WMTA, which is composed of nanoparticles ranging in size from 40–100 nm with several additives compared to WMTA. Nano WMTA exhibits remarkable advantages in the physiochemical properties including setting time, microhardness (12), compressive strength (13), resistance in acidic environments (14). However, the inherent angiogenic properties of these cements have not been previously determined.

There are a few studies regarding the angiogenic properties of calcium silicate-based cements. Huang et al. indicated that WMTA is able to activate the p38 pathway in human dental pulp stem cells (hDPSCs) (15). Yun *et al.* evaluated the combination effect of WMTA and growth hormone and indicated that this combination can promote angiogenesis events in hDPSCs (16). In a similar study, Chang et al. reported that the combination of WMTA and human

placental extract can promote angiogenesis in rat dental pulp (17). However, the inherent angiogenic properties of these cements have not been previously determined. The present study was undertaken to investigate the angiogenic properties of WMTA, Ca(OH)₂, Geristore, and Nano WMTA biomaterials. The hypothesis tested here is that these biomaterials exhibit different inherent angiogenic capacities that could impact their reparative potential.

Material and Methods:

The dental material including WMTA (ProRoot MTA, Dentsply, Tulsa, Okland, USA), Ca(OH)₂ (Calcium Hydroxide Powder USP, DHARMA Research, FL, USA), and Geristore (Den-Mat, Santa Maria, CA, USA) were prepared according to the suppliers directions. The angiogenic properties of these biomaterials were evaluated using in-vitro model of PEG hydrogel array (0.4 kPa shear modulus) similar to Matrigel, and EC isolated from mouse molar. For in-vivo experiments, the laser-induced mouse CNV model was used to assess the angiogenic activity of various biomaterials.

Isolation of EC from Dental Pulp:

The isolation of dental pulp was performed by a method used by Saghiri *et al.* (18, 19). Briefly, EC were isolated from one litter (6 or 7 pups) of 4-week-old C57BL/6j immort mice. Harvested molars were broken at the cement enamel junction (CEJ), and the pulp tissues were collected under a dissecting microscope. Pulp tissues were cut into small pieces using a razor blade and then digested in 5 mL of Collagenase type I enzyme (Worthington, Lakewood, NJ; 1 mg/mL in serum-free Dulbecco's modified Eagle's medium; DMEM) and incubated at 37°C for 40 min. The digested tissues were washed with DMEM containing 10% fetal bovine serum (FBS), and centrifuged. The pellet was re-suspended in 10 mL of DMEM with 10% FBS and passed through a nylon mesh with a pore size of 70 µm. The cells were pelleted and re-suspended in 1 mL of DMEM with 10% FBS and mixed with magnetic beads coated with PECAM-1 antibody prepared as previously described. The mixture allowed to rock at 4°C for 1 h. Following incubation, the beads were collected using a magnet and washed six times with 1 mL of DMEM with 10% FBS. The beads were then re-suspended in 0.5 mL of EC growth medium and plated in a well of a 24 well plate coated with fibronectin (2 µg/ml in serum free medium overnight) (20). The EC growth medium contained DMEM with 10% FBS, 2 mM L-glutamine, 2 mM sodium pyruvate, 20 mM HEPES, 1% non-essential amino acids, 100 µg/ml streptomycin, 100 U/ml penicillin, freshly added heparin at 55 U/ml (Sigma, St. Louis, MO), endothelial growth supplement 100 µg/ml (Sigma), and murine recombinant interferon-γ (R&D, Minneapolis, MN) at 44 units/ml. Cells were then passed to a 35 mm dish coated with 1% gelatin, and subsequently expanded in to gelatin-coated 60 mm dishes. The identity of EC was confirmed by FACS analysis for EC markers including CD31 and VE-cadherin. All cells were used prior to passage 15.

PEG Functionalization with Norbornene

Modification of PEG-OH molecules with terminal norbornene groups was performed using methods similar to previous studies. Briefly, PEG-OH (20 kDa molecular weight, 8-arm, trientaerythritol core, Jenkem USA, Allen TX), dimethylaminopyridine and pyridine

(Sigma Aldrich, St. Louis, MO) were dissolved in anhydrous dichloromethane (Fisher Scientific, Waltham, MA). In a separate reaction vessel, N,N'-dicyclohexylcarbodiimide (Thermo Scientific, Waltham, MA) and norbornene carboxylic acid (Sigma Aldrich) were dissolved in anhydrous dichloromethane and reacted for 30 minutes to activate the norbornene. Norbornene carboxylic acid was covalently coupled to the PEG-OH through the carboxyl group by combining the PEG solution and norbornene solutions and stirring the reaction mixture overnight under anhydrous conditions. Urea byproduct was removed from the reaction mixture using a glass fritted funnel and the filtrate was precipitated in cold diethyl ether (Thermo Fisher Scientific, Inc., Carlsbad, CA) to extract the norbornene-functionalized PEG (PEGNB). The precipitated PEGNB was collected and dried overnight in a ceramic fritted filter. To remove impurities, the PEGNB was dissolved in chloroform (Sigma Aldrich), precipitated in diethyl ether and dried a second time in a buchner funnel. To remove excess norbornene carboxylic acid, PEGNB was dissolved in de-ionized H₂O, dialyzed in de-ionized H₂O for 1 week and filtered through a Millex® 0.45 µm pore-size PVDF syringe filter (Millipore). The aqueous PEGNB solution was frozen using liquid nitrogen and lyophilized. Functionalization of PEG was quantified using proton nuclear magnetic resonance spectroscopy (NMR) to detect protons of the norbornene-associated alkene groups located at 6.8–7.2 PPM. Functionalization efficiency for norbornene coupling to PEG-OH arms was above 90% for all PEGNB used in these experiments.

Formation of the PEG Hydrogels:

Hydrogel substrates were formulated and characterized in work by Nguyen *et al.* (21), and hydrogel substrates were formed here using similar methods. Briefly, hydrogel solutions, containing as 2 mM of 20 kDa, 8-arm norbornene-functionalized PEG, 0.125 mM cyclized RGD peptide (RGDfC, Genscript, Piscataway, NJ), and 4 mM crosslinking peptide (KCGGPQGIWGQGCK, Genscript) and 0.2% I2959 photoinitiator (Ciba Specialty Chemicals, Tarrytown, NY) dissolved in 1X PBS, were added as 100 µL volumes to 48-well plates the droplets were cross linked under 365 nm UV light for 2 min at a dose rate of 4.5 mW/cm². MiliQ water (300 µL) was added on top of the hydrogels to hydrate the gels prior to seeding the cells (Fig 2).

Sample Elution Preparation:

For PEG hydrogel array and CNV studies, an elution of the tested materials was prepared. The powder and liquid of WMTA (ProRoot MTA, Dentsply, Tulsa, Okland, USA), Ca(OH)₂ (Calcium Hydroxide Powder USP, DHARMA Research, FL, USA), and Geristore (Den-Mat, Santa Maria, CA, USA) were prepared and mixed according to the manufacturer's instruction, and molded in to a 2×2×2 mm cube and after 1day incubation. The cubes were removed aseptically and dispersed into 2 mL MiliQ distilled water for 2 days, centrifuged, and the 2 mL supernatant medium was collected. The elution prepared from each sample was subjected to EC tube formation on hydrogel arrays and laser-induced CNV as described below.

PEG Hydrogel Tubulogenesis assays:

All hydrogel wells were seeded with molar EC at 2,500 cells/cm² for 12 h. At the time of plating, cells were mixed with elution prepared from each sample at a ratio of 3:1 (3 parts

medium: 1 part elution material) and saline was used as control. The density of cells for each experimental group was compared to a medium control. The cocktail of medium and elution of tested biomaterials in ratio of 3:1 was replaced every 24 h. After 72 h of culture, the arrays were washed with serum-free medium and stained with a 5 μ M Cell Tracker Green (Invitrogen) solution prepared serum-free medium for 45 min. After 15 min of staining, the arrays were washed with serum-free medium and incubated for 30 min in growth medium. The arrays were then washed with PBS and fixed for 30 min in 10% buffered formalin (Fisher). The wells were incubated in PBS overnight and photographed using a Nikon TE300 fluorescence microscope within 48 h of fixation.

Image Analysis:

There are several different ways to detect and quantify tube network formation. The most common methods of analysis involve quantification of number of tubes, nodes, loops/meshes, or length of tubes. These parameters can be counted by hand or can be done through various imaging programs, including NIH ImageJ with the angiogenesis analyzer plugin (National Institutes of Health, USA). Counts of number of nodes and total branch length of tubulogenesis were done automatically. The raw images were opened and converted into 8-bit file, smoothed by the unsharp mask feature (settings 1:0.2) and background removed (rolling ball radius 10). The resulting images were then converted to binary images by setting a same threshold. The threshold values were determined empirically by selecting a setting which gave most accurate binary images. The cell area was determined by manual delineation on raw fluorescent images.

CNV Model and Treatment with Vital Pulp Therapy Materials:

The second model utilized in this study was the laser-induced mouse CNV model as previously described by us (22). The animal studies were carried out in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin School of Medicine and Public Health. CNV was induced in C57BL/6J mice (Jackson Laboratories; 6-weeks old female) by laser photocoagulation-induced rupture of Bruch's membrane on day 0. Mice were anesthetized with ketamine hydrochloride and xylazine, and pupils were dilated using tropicamide ophthalmic solution. Laser photocoagulation (75 mm spot size, 0.1 s duration, 120 mW) was performed in the 9, 12, and 3 o'clock positions of the posterior pole of each eye with the slit lamp delivery system of an Oculight GL diode laser (Iridex) and a handheld cover slip as a contact lens to view the retina. The prepared extracts of WMTA, Ca(OH)₂, Geristore, Nano WMTA and control were delivered by intravitreal injection (2 μ L/eye) at day of laser and one week later.

The Evaluations of CNV:

After 14 days, the eyes were removed and fixed in 4% paraformaldehyde at 4°C for 2 h. Following 3 washes in PBS, the eyes were sectioned at the equator, and the anterior half, vitreous, and retinas were removed. The remaining eye tissue were incubated in blocking buffer (5% fetal calf serum and 20% normal goat serum in PBS) for 1 h at room temperature, followed by incubation with anti-ICAM-2 (1:500 in PBS containing 20% fetal

calf serum, 20% normal goat serum; BD Biosciences) overnight at 4°C. The remaining eye tissue were then washed 3 times with PBS and incubated with the appropriate secondary antibody. The retinal pigment epithelium-choroid-sclera complex were dissected through 5 to 6 relaxing radial incisions and flat mounted on a slide with Vecta Mount AQ (Vector Laboratories, Inc.). The samples were viewed by fluorescence microscopy and images were captured in digital format using a Zeiss microscope (Zeiss). ImageJ software was used to measure the total area (in μm^2) of CNV associated with each burn. This part of image processing is similar to Saghiri *et al.* (23). Briefly Images were opened, inverted, set scaled and converted into 8-bit file. Eventually smoothed by the unsharp mask feature and count the area in μm^2 . The resulting images were then converted to binary images by setting a same threshold.

Statistical Analysis: A one-way ANOVA and Tukey post-hoc statistical tests were used to detect any statistically significant differences among the values of number of nodes and total branch length in experimental groups at level of significance $p < 0.05$.

Results:

Fluorescence activated cell sorting (FACS)

Endothelial cells we checked for the expression of various EC markers including PECAM-1/CD31, VE-cadherin, and B4-lectin by FACS analysis. All EC expressed significant amounts of these markers. (Fig. 1).

Tubulogenesis Image Processing and Number of Nodes:

Angiogenic activity of endothelial cells in the PEG-based tubulogenesis assay, measured using the number of nodes counted in endothelial cell networks, was diminished by treatment with dental materials tested here. The means and standard deviation of number of nodes for control and experimental groups were: control group = 316.00 ± 60.48 , Geristore = 289.00 ± 129.52 , Nano WMTA = 297.71 ± 61.14 , WMTA = 106.33 ± 33.00 , and $\text{Ca}(\text{OH})_2$ = 64.14 ± 19.32 . The values of Geristore, Nano WMTA, and control groups were not significantly different from each other ($P > 0.05$), while there were significantly different from WMTA and $\text{Ca}(\text{OH})_2$ ($p < 0.05$). There was no significant difference between WMTA and $\text{Ca}(\text{OH})_2$ ($p > 0.05$) (Fig. 3A). There were no outliers and the data were normally distributed for each group, as assessed by box plot and Shapiro-Wilk test ($p > 0.05$), respectively. Homogeneity of variances was violated, as assessed by Levene's Test of Homogeneity of Variance ($p < 0.05$). The score of "number of nodes" was significantly different for different material groups, Welch's $F(4, 13.294) = 43.441$, ($p < 0.05$).

Tubulogenesis Image Analysis and Total Branch Length:

Using an alternative metric of total branch length of endothelial cell networks in the tubulogenesis assay (μm), upregulation of angiogenic activity of endothelial cells was detected after treatment with Geristore. The means and standard deviation of total branch length of control and experimental groups were: control group = 1987.57 ± 87.13 , Geristore = 2011.42 ± 663.16 , Nano WMTA = 1485.14 ± 168.48 , WMTA = 1121.83 ± 171.79 , and

$\text{Ca(OH)}_2 = 581.42 \pm 155.01$). The value of Ca(OH)_2 was significantly different than all other groups ($p < 0.05$).

Geristore and control groups showed significantly different values than other groups ($p < 0.05$), while there were not significantly different from each other ($p > 0.05$). Results of WMTA and Nano WMTA did not show any significant difference from each other ($p > 0.05$), while they were significantly different than Ca(OH)_2 ($p < 0.05$), Geristore, and control groups ($p < 0.05$) (Fig. 3B). There were no outliers, as assessed by box plot. The data were normally distributed for each group, as assessed by Shapiro-Wilk test ($p > 0.05$). There was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p > 0.05$). Total branch length was significantly different between experimental groups, $F(4, 29) = 135.339$, ($p < 0.05$).

CNV Analysis:

To begin assessing the impact of dental materials on angiogenesis *in vivo*, blood vessels were treated with dental material elutions in CNV models. Here Geristore treatment enhanced angiogenic activity (Fig. 4A), and the impact of other treatment groups followed similar trends shown in the total branch length measurements taken in the tubulogenesis assay. The means and standard deviations of control and experimental groups were: control = $1175.57 \pm 410.06 \mu\text{m}^2$, Geristore = $1350.57 \pm 282.46 \mu\text{m}^2$, Nano WMTA = $1122.85 \pm 410.06 \mu\text{m}^2$, WMTA = $784.83 \pm 354.60 \mu\text{m}^2$, and $\text{Ca(OH)}_2 = 616 \pm 226.78 \mu\text{m}^2$." However, the only significant difference was noticed between the results of Geristore and control groups with Ca(OH)_2 group ($p < 0.05$) (Fig. 4B). There were no outliers, as assessed by boxplot. The data were normally distributed for each group, as assessed by Shapiro-Wilk test ($p > 0.05$). There was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p > 0.05$). CNV score was significantly different between different material groups, $F(4, 29) = 5.277$, ($p < 0.05$).

Discussion:

A number of the commonly used vital pulp therapy materials were used for evaluation of their angiogenic bioactivity through an in-vitro model of PEG hydrogel arrays and in vivo mouse model of laser-induced CNV. The use of non-fouling hydrogels as a cell culture substrate enables investigations on how endodontic biomaterials affect angiogenesis while minimizing extraneous cell signaling modalities that potentially mask the effects of the endodontic biomaterials. The PEG hydrogels utilized in these studies were designed to present specific cell adhesion molecules and mechanical stiffness to EC and direct them to form capillary-like tubule networks in two-dimensional culture (21). According to the fact that EC have the most important role during angiogenesis, these cells were isolated from pulp tissue and considered in in-vitro studies (24, 25).

Matrigel is a complex biomaterial that is composed of thousands of proteins and biomolecules with numerous limitations including batch-to-batch variation, poor stability and handling properties, and confounding components including matrix-bound growth factors that can decrease the sensitivity and consistency of tubulogenesis assays. The use of a chemically-defined synthetic hydrogel was expected to circumvent the limitations of

Matrigel and has demonstrated the ability to increase the consistency and sensitivity of the tubulogenesis assays (21), and was therefore, utilized in the current studies.

The results of present study showed that all tested materials diminished angiogenesis except Geristore, which exhibited a pro-angiogenic behavior compared to the control group. $\text{Ca}(\text{OH})_2$ was one of the first material used for vital pulp therapy procedures. The release of Ca and high pH value was shown to be the reason for bioactivity of $\text{Ca}(\text{OH})_2$. It has two main effects including the antibacterial activity due to its alkalinity, and triggering the dentin bridge formation due to the calcium (Ca) ion release at the applied site (26). However, previously it was reported that the limited angiogenic activity of $\text{Ca}(\text{OH})_2$ is attributed to the release of growth factors preserved in dentin matrix such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor β (TGF- β), and insulin-like growth factor (IGF) (9). These outcomes were consistent with present study results as $\text{Ca}(\text{OH})_2$ showed the least value in number of EC nodes, vascular total branch length, and neovascularization in the CNV model.

WMTA group also showed decreased angiogenic activity in both hydrogel array and CNV studies compared to the control group. However, it was noticed that WMTA was able to promote angiogenesis more than $\text{Ca}(\text{OH})_2$. This difference between the behavior of WMTA and $\text{Ca}(\text{OH})_2$ can be explained by the pro-angiogenic activity of WMTA. It was reported that WMTA can increase the release of pro-angiogenic factors such as VEGF, interleukin-1 β , interleukin-8, TGF- β 1, and inducible nitric oxide synthase (iNOS) (1). In addition, it was indicated that WMTA can enhance the expression of angiopoietin-1 and von Willebrand factor, which resulted in activation of the mitogen-activated protein kinase (MAPK) signaling pathways in human dental pulp stem cells (15). Although WMTA presents some direct pro-angiogenic activities, it seems that these effects are limited as previous authors attempted to increase angiogenic properties of WMTA. Yun *et al.* used WMTA in combination with growth hormone (GH) in order to enhance the angiogenic activity of this calcium silicate-based cement. It was concluded that the combination of WMTA and GH could induce angiogenesis due to the synergic proangiogenic effect of GH through the BMP-2 pathway (16). Similar results were noticed in the present study, as WMTA showed more angiogenic bioactivity than $\text{Ca}(\text{OH})_2$, while these characteristics were lower than Geristore and Nano WMTA. Although several aspects of this cement were evaluated, its angiogenic activity remained unclear. Although Nano WMTA exhibited decreased angiogenesis, but it promoted angiogenesis significantly more than WMTA and $\text{Ca}(\text{OH})_2$. This can be explained, at least in part, by the differences in composition of these cements.

Zeolite, also known as clinoptilolite, is one of the most important additives of Nano WMTA cement. It is a hydrated aluminosilicate crystalline with anti-corrosive behavior that is added to the cement to produce a more stable and corrosion resistant material (27). Beside corrosive inhibitory effects, it was reported that zeolite has a high restoring capacity for nitric oxide (NO) (28). It is evident that NO can function as an autocrine regulator of the microvascular events necessary for neovascularization and mediates angiogenesis (29). Angiogenesis events are initiated with vasodilation, and NO is a factor responsible for vasodilatation in physiological and pathological angiogenesis (30). NO significantly contributes to the pro-angiogenic events of capillary endothelium by inducing the expression

of cell growth and differentiation (30). NO pathway was suggested to be a promising target for considering the pro- and anti-angiogenic therapeutic strategies (30). These findings about the characteristics of zeolite might explain the better pro-angiogenic bioactivity of Nano WMTA cement compared with WMTA and $\text{Ca}(\text{OH})_2$ (Fig. 4 A, B).

Among all tested materials, Geristore was the only biomaterial that presents proangiogenic behavior compared with the control group, while other tested biomaterials decreased angiogenesis. This superior pro-angiogenic effect might be explained by the alumina silica content, which is also found in the structure of zeolite in Nano WMTA cement. In addition to alumina silica glass, it was shown that methacrylic acid (MAA) can enhance angiogenesis and wound closure through modification of gene expression (31). In another study, the butyl methacrylate (BMA) scaffolds were co-polymerized with MAA and implanted subcutaneously in mice. The histological analysis showed a significant enhancement of angiogenesis and capillary density in samples with BMA-MAA scaffolds (32). Thus, the combination of alumina silica glass and MAA in Geristore may be responsible for the superior pro-angiogenic behavior of this biomaterial.

Conclusion:

- According to the outcomes of the present study the hypothesis was accepted as tested dental materials exhibit different angiogenic potential.
- It was concluded that only Geristore has direct pro-angiogenic activities, while the rest of the materials tested appeared to diminish angiogenic activity to varying degrees compared to the control.
- Future studies are required to evaluate the angiogenic and dentinogenic behavior of tested dental materials regarding dental pulp tissue in pre-clinical models.

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Statement of Clinical Relevance:

Regarding the angiogenic properties of dental pulp capping materials, only Geristore demonstrated enhanced angiogenic activity, while other tested calcium silicate-based cements had diminished angiogenic activity. Base on our findings here, Geristore can be regarded as a proangiogenic dental pulp capping material, which can be used in the cases where enhancement of angiogenesis events is required at the applied area.

Figure 1. Pulp endothelial cells expressed various EC markers including PECAM-1/CD31, VE-cadherin, and B4-lectin by FACS analysis.

Figure 2.

A) Schematic representation of hydrogel formation. B) Cross-linked hydrogels appearance.

Figure 3.

Box plots of means and standard deviations of number of nodes (A) and total branch length of WMTA (white), Ca(OH)₂ (blue), Geristore (green), Nano WMTA (red), and control (yellow) groups.

Figure 4.

A) Schematic presentation of CNV model and image processing of Laser lesions (up). The image of blood vessel network formation of experimental groups, which showed reduced blood vessel network formation in Ca(OH)_2 and WMTA samples in mouse CNV model. Representative sclera-choroidal flatmounts after staining with intercellular adhesion molecule-2 antibody; CNV in mice that received WMTA, Ca(OH)_2 , Geristore, and Nano WMTA (bottom left). Sample of laser-induced bilateral vitreous hemorrhages (bottom right); B) Box plots of means and standard deviations of lesion areas (μm^2) in the CNV model for WMTA (white), Ca(OH)_2 (blue), Geristore (green), Nano WMTA (red), and control (yellow) groups.