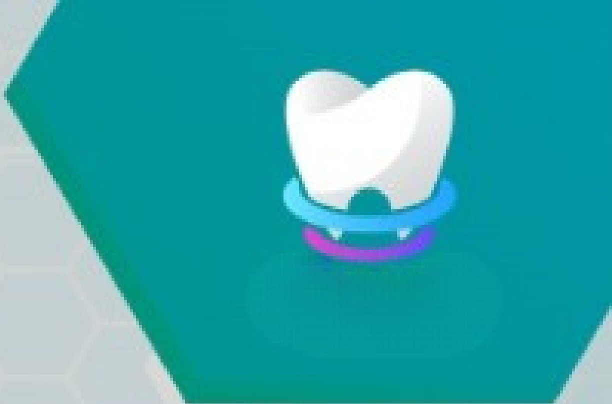

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Effect of composite resin monomer BisGMA on odontogenic differentiation of human dental pulp stem cells

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ABSTRACT

Objective : To study the effect of composite resin monomer BisGMA on human dental pulp stem cell (hDPSCs) odontoblasts differentiation, and explore the effect of composite resin filling teeth on dentin formation.

Material and Methods : separate and culture hDPSCs, identify cell phenotype by flow cytometry, and induce staining to assess multidirectional differentiation potential. The hDPSCs was stimulated with different concentrations of monomeric BisGMA, and the odontoblast differentiation capacity of each group was determined by CCK-8 assay, alkaline phosphatase activity detection, and q-PCR. Results: CCK-8 showed that the addition of 0.5 mM —— 8 mM BisGMA affected the cell increment, but no significant difference; the concentration of 0.5 mM —— 8 mM BisGMA reduced the cellular alkaline phosphatase activity and further decreased the activity with increasing concentration.The q-PCR results also showed that monomeric BisGMA inhibited the dentin-related gene OC (osteocalcin), DSPP (dentin sialophosphoprotein)Expression, and with the concentration increases, the inhibition is more obvious of.

Conclusion : Composite resin monomer BisGMA has a negative effect on odontogenic differentiation of human dental pulp stem cells.

Keywords: *BisGMA; human dental pulp stem cells; odontinal differentiation*

INTRODUCTION

Odontoblasts, located around the pulp, the main function is to form the dentin. However, because odontoblasts are terminally differentiated cells, immortalized oblasts cannot be cultured. At present, the function and molecular mechanisms of dental pulp stem cells are mainly studied by inducing the differentiation of dental pulp stem cells (DPSCs) to odontoblast cells. Due to the existence of a stem cell population in the pulp tissue, the development of the regeneration of tissue engineering technology.

Bisphenol A dimethacrylate (bisphenol A digly-cidyl methacrylate, BisGMA) is one of the more commonly used resin matrix. At present, more than 70% of the filling composite resin has Bis-GMA as the main matrix on the market. Bis-GMA is the main monomer component produced by incomplete composite resin polymerization. In this study, monomers with different low concentrations of Bis-GMA were used to be cocultured with human dental pulp cells to observe the effect of Bis-GMA on odontoblastic differentiation of human dental pulp cells.

MATERIALS & METHODS

Reagents

Bisphenol A dimethacrylate (bisphenol A digly-cidyl methacrylate, BisGMA)were obtained from Sigma Aldrich (Taufkirchen, Germany).DMEM medium (Gibco, USA); Fetal bovine serum (Gibco, USA); 0.25% tryptase (Gibco, USA); PBS solution (Key, China);Alkaline phosphatase (AKP) kit: Nanjing Jiancheng Bioengineering Institute, A059-2;Trizol Reagent: Lifetech 15596026;Primer design: Beacon Designer 7.90;Primer synthesis: invitrogen, Guangzhou

Histological sample preparation

Cell culture and identification: Using frozen human pulp stem cells. After the primary pulp cells are expanded, the cells were transferred to 10cm dishes. The medium was changed once every 3d, and cells from passages 3 to 5 were used for further experiments.hDPSCs Phenotype was identified using flow cytometry to identify the hDPSCs phenotype.

The effect of BisGMA on hDPSCs proliferation was assessed using CCK-8. Generation 3 hDPSCs was seeded at a density of 3000 cells / well in then 96-well plate. When cells were

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attached for 24h, change with complete medium containing different concentrations of BisGMA (0.5 mM, 1 mM, 2 mM, 4 mM, 8 mM, 16 mM,), change the medium for 2-3d, 5 complex wells were set for each group of experiments, after 14d of induction, CCK-8 detection: Effect of BisGMA on hDPSCs differentiation:Will generation 3 hDPSCs Cells were seeded in 6-well plates at a density of 105 cells / well. Cell paste Wall for 24h, were stimulated with different concentrations of BisGMA, and cells were grown to At 80% confluence, the induction medium was added. Odontinal induction medium (DMEM + 10% FBS, 10 mmol/L β -sodium glycerophosphate, 50g / L ascorbic acid). Assays were performed on days 3 and 7 of induction. Cells were lysed using protein lysate, and the supernatant was collected by centrifugation and gently shaken well plates were mixed at 520nm. The absorbance OD of each well was measured in a microplate reader. The calculation formula of the culture medium is defined: 100 mL liquid at 37°C and matrix for 15min to produce 1mg phenol as 1 Kim unit.computational formula: AKP viability (Kim unit / 100 mL) = (measured OD-blank OD) / (standard OD-blank OD) Standard concentration (0.02 mg/mL) pretest dilution of 100 mL sample q-PCR:Generation 3 hDPSCs was seeded in 6-well plates at a density of 105 cells / well, and total RNA was collected using TRIzol 7d after BisGMA stimulation, and equal amounts of RNA were reverse transcribed into cDNA., An equal amount of RNA was reverse-transcribed into cDNA. Using SYBR Green Supermix in the same way Stepone™ The real-time PCR system performed real-time quantitative PCR on cDNA as template to detect the expression of dentin-related gene OC (osteocalcin), DSPP (dentin sialophosphoprotein) in hDPSCs, with actin (β -actin) as the internal reference. exploitation $2^{-\Delta\Delta ct}$ Methods Analysis of gene expression levels. The primer sequences used are shown in Table 1

Table 1 : The q-PCR primers and their sequences

Sequence	Name	Length(bp)
TTGCCCTCAACGACCACTTT	GAPDH(H)-F	120
TGGTCCAGGGGTCTTACTCC	GAPDH(H)-R	
CAGAGTCCAGCAAAGGTG	OC (H)-F	88
AGCCATTGATACAGGTAGC	OC (H)-R	
CAGTGATAGCAGTGACAG	DSPP (H)-F	78
TTGTTGTTACCGTTACCA	DSPP (H)-R	

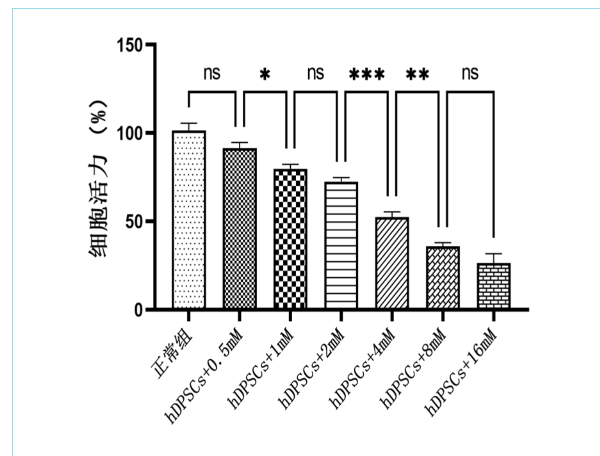
Statistical analysis

Two-group t-tests were performed using GraphPadPris5 software, one-way ANOvariance tests with fully randomized design between multiple groups, and differences were considered statistically significant at P <0.05.

RESULTS

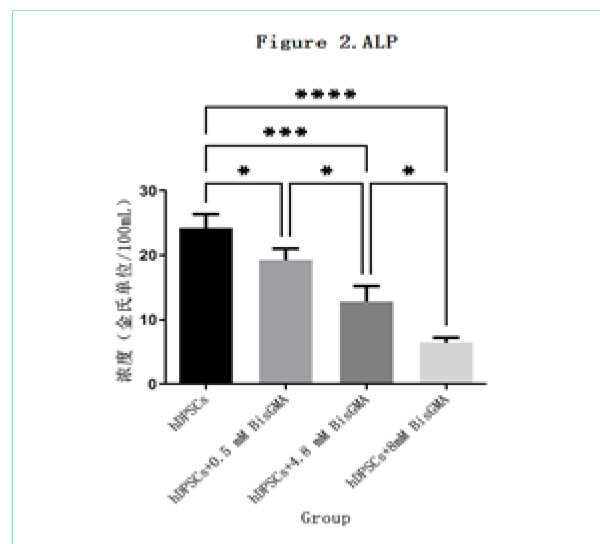
2CCK 8 results showed that compared with normal cells, cell viability decreased significantly and was negatively correlated with BisGMA concentration. hDPSC cells and inhibited hDPSC cellswere stained with Alizinarin see Figure 5 and Figure 6. The cytoinhibition rate calculated from the CCK 8 experiment, Using the SPSS statistical software, The Regression-probit regression regression BisGMA concentration against cytostatic rate, The half inhibitory concentration of BisGMA IC50, The results are as follows: According to the analysis, The addition of 0.5 mM BisGMA affected the cells, However, there was no significant difference; There was no significant difference between the addition of 8 mM BisGMA and 16 mM BisGMA; Therefore, experiments with a minimum concentration of 0.5 mM, The highest concentration of 8 mM BisGMA, The IC50 was 4.805 mM.(Figure .1)

Figure 1.CCK8



Results of the alkaline phosphatase activity:BisGMA did not affect hDPSCs alkaline phosphatase viability at a concentration of 0.5 mM but showed inhibition with increasing concentration Compared with the control, Significance (P <0.05).(Figure. 2)

Figure. 2



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Results of the OC and DSPP q-PCR:hDPSCs After 7d of culture under different BisGMA stimulation, the odontogenesis-related gene OC (oste`ocalcin) and DSPP (dentin sialophosphoprotein) mRNA expression were inhibited, and the inhibition was stronger with increasing concentration. The results are shown in Figure 3 and Figure 4.

Figure 3

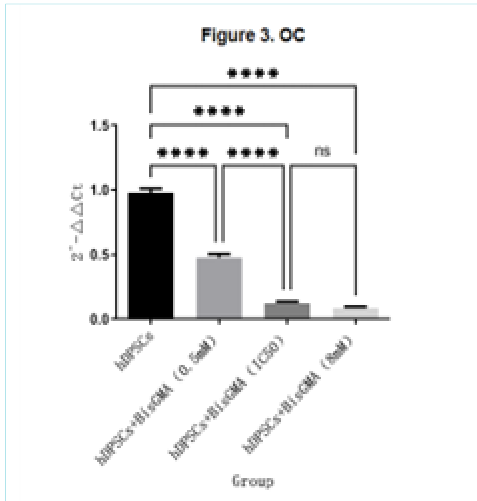

Figure 4

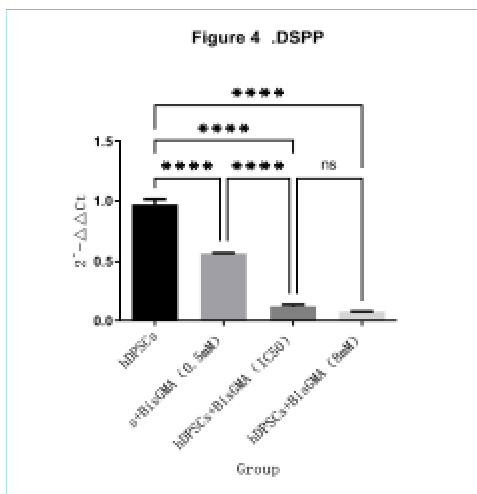

Figure 5

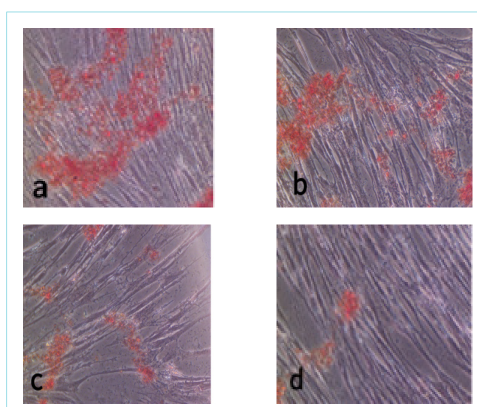

Figure 5.hDPSC cells were stained with Alizinarin for 200X(a) BisGMA hDPSCs

(b)hDPSCs+0.5 mM BisGMA (c)hDPSCs+4.8 mM BisGMA (d):hDPSCs+8 mM BisGMA:the mineralized nodules stained with alizarin red gradually decreased

The results of Alizarin Red S Staining showed that with the BisGMA concentration from low to high, the mineralized nodules stained with alizarin red gradually decreased (Figure a~d)

DISCUSSION

Clinically, the composite resin is used for filling treatment, and some monomers will remain after the polymerization of the composite resin, the main components are bisphenol A diglycidyl methacrylate (BisGMA), triethylene glycol dimethacrylate (TEGDMA), Polymethacrylate methyl vinegar and carbamic acid acetate bismethacrylate, etc., these unreacted residual monomers can release free radicals or directly damage tissues after entering the mouth, dentin, and apical tissues. Although not in direct contact with the pulp, it may affect dentinocytes. The dentinocytes in the pulp tissue are located on the periphery of the pulp tissue, and the odontoblasts are arranged close to the dentin wall, and their protrusions often penetrate deep into the dentin tubules. When chemical and physical stimulation acts on dentin, it can be conducted to the pulp through the dentin fluid in the dentin tubule, the pulp responds, the undifferentiated mesenchymal stem cells in the pulp are activated, differentiate into dentinocytes, secrete dentin matrix, form the third stage dentin, including reactive dentin and restorative dentin, protect the pulp, this is the pulp-dentin complex, in response to stimulation, It plays a pivotal role in maintaining the health of dental tissue. Since odontoblasts are terminally differentiated cells, immortalized culture is not possible. At present, the functional and molecular mechanisms of dentalpulp stem cells (DPSCs) are mainly studied by inducing the differentiation of dentalpulp stem cells into odontoblasts. Dental pulp stem cells are activated after being stimulated by the apoptosis signal of odontoblasts, and then proliferate, migrate and differentiate into odontoblasts to form restorative dentin. hDPSCs are ectoderm-derived mesenchymal stem cells with multi-lineage differentiation potential, which can differentiate into chondrocytes, adipocytes, dentinocytes and neuro-like cells (dentalpulp stem ce11s, DPSCs) can form an ectopic pulp dentinin complex-like structure in nude mice, hence the name pulp stem cells. There are many studies on cytotoxicity at home and abroad, such as composite resin monomers can cause different degrees of damage to pulp and periodontal tissues by interfering with the redox system balance of cells,

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inhibiting phospholipid phthaloinositol-3 monokinase and protease-B signaling pathway, activating mitogen-activated protease signaling pathway, inducing the expression of inflammatory factors and promoting inflammatory response, regulating cell cycle checkpoints, inhibiting cell cycle, etc. In this study, the effect of composite resin monomer BisGMA on DPSCs was discussed, and the effect of monomers on dentin differentiation of DPSCs was initially understood at the cellular level, and then the effect of pulp-dentin complex on the formation of tertiary dentin by monomer stimulation was discussed. DSPP (dentin sialophosphoprotein), a non-collagen extracellular matrix protein secreted by odontoblasts, plays an important role in the regulation of odontoblast differentiation and dentin mineralization, and can be used as a specific marker of dentinocytes. The detection of OC (osteocalcin) was further supplemented to observe the effects of osteogenic differentiation; Alkalinephosphatase (ALP) is also an early marker of dentin differentiation and is closely related to dentin formation. In this study, the differentiation of DPSCs into odontoblast-like cells was induced in vitro, and the effect of composite resin monomer on the dentin differentiation of pulp cells was detected, and the experiment revealed that this effect was negative, and BisGMA monomer had an effect on the relevant indicators of dentin differentiation at a concentration of 0.5mM. In this study, ALP staining was performed on different BisGMA monomer concentration groups, and it was found that with the increase of monomer concentration, the level of ALP activity was significantly weakened. In q-PCR experiments, the expression of dentin/osteogenesis-related genes DSPP (dentin sialophosphoprotein) and OC (osteocalcin) was inhibited, and the inhibition became stronger with increasing concentration. Similarly, with the increase of the concentration of monomers, the results of alizarin red staining also showed that the mineralized nodules of alizarin red staining gradually decreased, indicating that the degree of mineralization was affected.In summary, composite resin monomers can affect the activity of odontoblasts and inhibit the ability of dentinocytes to differentiate into dentin, which may explain the clinical reasons for pulp inflammation or necrosis in some composite resin filling deep cavities.

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