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Research Article

Biocompatibility of Dentin-Pulp Membrane (BBio) with Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

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Abstract

This study aimed to evaluate the biocompatibility of dentin-pulp biostimulation membrane (BBio) with Stem cell from Human Exfoliated Deciduous teeth (SHED). SHED were obtained from primary culture and stored in biobank. Material extracts were prepared with αMEM 10% Fetal Bovine Serum (FBS) according to the following groups: Group 1 (G1) - BBio; Group 2 (G2) - Biodentine; and Group 3 (G3) - MTA Repair HP. Positive (C+) and negative control (C-) groups were respectively maintained in αMEM + 10% FBS and αMEM + 1% FBS. MTT assay evaluated cell viability, after 24, 48 and 72 hours of SHED contact with the extracts. Data were analyzed by two-way ANOVA followed by Tukey test ($p<0.05$). The treatment and period comparisons showed statistically significant differences ($p<0.000$). Intergroup comparison showed greater cell viability for G3. The BBio biocompatibility with SHED was similar to that of other pulp capping materials available in dental market.

Keywords: Stem Cell; Stem Cell Research; Cell Viability; Materials Testing; Biocompatible Materials

Introduction

Biomaterials are natural or synthetic compounds joined to biological systems to repair or replace cells, tissues and organs, contributing for the individual's quality of life. Biocompatibility is the capacity of a material to make its function without causing any side effect but allowing a favorable cellular and tissue response [1]. Thus, the search for new biomaterials with bioactive properties, biodegradability and nontoxicity is constant [2-4]. Experimental and clinical studies aimed at developing materials with desirable properties to totally or partially replace lost biological tissues or organs damaged by pathology or trauma, as close as possible the ideal anatomy and function of the involved area [5]. Therefore, the current tissue bioengineering tendencies are either to replace injured cells or to stimulate cell repair and growth through biomaterials [6].

Studies point out the biochemical and biophysical characteristics of membrane's immense potential for bioengineering application in dentistry. In this context, the new dentin-pulp membrane (BBio) is a three-layered chitosan-based membrane that enabled the incorporation of many materials [6-9]. The search for new biomaterials with bioactive properties, biodegradability and non-toxicity is ongoing. Since current research must verify the cellular and molecular efficacy of the biomaterials under development, this study opens new treatment perspectives for pulp therapy in primary teeth [10-22]. Studies point out the biochemical and biophysical characteristics of membrane's immense potential for bioengineering application in dentistry. In this context, the new dentin-pulp membrane (BBio) is a three-layered chitosan-based membrane that enabled the incorporation of many materials and meets the global trend of development, research and sustainability [6-9].

Chitosan is indicated for biological procedures because it is a natural polymer, nontoxic, biocompatible, biodegradable, bactericidal and hemostatic, with tissue regeneration activity [10]. Its action is associated with tissue healing through cell proliferation, biomodulation of collagen structures and immunomodulation activity resulting in anti-inflammatory action. Because of favorable capacity of stimulating pulp cells, chitosan has been associated with other materials to produce new bioactive pulp capping materials. The association of chitosan with tricalcium cement may optimize and potentialize the properties of both materials with easy handling and low cost [6,8].

Vital pulp therapy goal is to regenerate the dentin-pulp complex of primary teeth up to exfoliation. The literature indicates that SHED, obtained from the pulp of exfoliated human deciduous teeth, are a highly proliferative, multipotential population with the capacity to differentiate into several cell types, such as neural cells, adipocytes, osteoblasts and odontoblasts. Therefore, the materials should induce pulp cell proliferation through biostimulation, release calcium ions, boost electroconductivity, produce hydrogen and enable the formation of apatite crystal layer between the material and the dentin [6,11]. Thus, this study aimed to evaluate the biocompatibility of dentin-pulp Biostimulation membrane (BBio) with Stem Cell from Human Exfoliated Deciduous teeth (SHED).

Methods

BBio Construction

A three-layered chitosan-based membrane was constructed associated with calcium silicate cement (chitosan/cement/chitosan). The proportions of each material were pre-determined and followed previous studies 6-9. 60X15 mm Petri plates were used as mold. Each layer was individually placed and dried at 30°C for 15 minutes. Then, the plates were let to drying for 24 hours inside an oven. After that, the membranes were cut with the aid of round metallic puncher (diameter = 5 mm; height = 3 mm) and sterilized by ultraviolet light for one hour inside a biological safety booth [6,8].

Sample Preparation

The following three biomaterials were assessed in this study: BBio, Biodentine. The material extracts preparations were performed according to previous studies and ISO 10993 guidelines [12-15]. BBio was previously prepared as described above and the cements were mixed according to the manufacturer's instructions. The cement samples were obtained in sterile conditions by using sterile rubber molds (5 mm diameter, 3 mm height) and incubated at 37 °C for 6 h. Elapsed that time, the samples were removed from the molds and sterilized by ultraviolet light for 1h inside biosafety cabinets. Each sample was immersed into 1 mL of αMEM + 10% FSB + 1% antibiotics and antifungals and incubated at 5% CO_2 for 3 days. Elapsed that period, the material discs were discarded and the supernatants were collected and filtered with 0.22 mm sterile filter.The collected supernatants were referred as extracts (1:1). The treatments were evaluated as follows: Group 1 - BBio; Group 2 - Biodentine; Group 3 - MTA Repair HP; Group 4 -Positive control (α-MEM+10% FSB); Grupo 5 -Negative control (α- MEM+ 1% FSB), at the respective time periods of 24, 48 and 72 h [12-15].

Cell Viability Assay

SHED at 7th passage were cultivated in αMEM supplemented with 10% fetal bovine serum and 1 % antifungals+antimicrobials, at 37 °C, 100% humidity, 95% air and 5% CO2. Culture medium was changed periodically until reaching subconfluence inside culture flask. Cell viability was analyzed by MTT assay after 24, 48 and 72 h of SHED contact with the extracts. SHED at density of 1 x 104/ 100µl of medium were seeded in 96-well plates (Corning #3595) and incubated for 24 h, at 37°C and 5% CO_2 for cell adhesion. After that period, the culture medium was changed by the materials extracts following the study periods. Elapsed the study times, the culture medium was removed and the cells were washed with PBS 1x. Next, 110µl of MTT (0.5 mg/mL) was added to each well. The plates were covered with aluminum foil and incubated at 37 °C, 5% CO_2, for 4 h. After that, MTT solution was discarded and 200 ul of Dimethyl sulfoxide were added per well. After 30 minutes, absorbance was measured by spectrophotometer at 570 nm wavelength. Groups were plated in triplicate and the cell viability assay were analyzed in three independent experiments [21].

Data Analysis

All statistical analyses were obtained with Statistica 10.0 software for Windows. Data were analyzed by two-way ANOVA followed by Tukey test. All values were presented as mean ± Standard Deviation (SD).

Results

The treatment and period comparisons (24, 48 and 72 h) showed statistically significant differences ($p<0.05$). At 24 h, the intergroup comparison showed the following cell viability values G3>G1>G2 ($p<0.05$). At 48 h, MTA Repair HP showed statistically greater cell viability values than both BBio and Biodentine ($p<0.05$). At 72 h, BBio showed statistically greater cell viability values than Biodentine, but smaller values than MTA Repair HP. Positive Control (C+) showed the highest cell viability values, with statistically significant differences (Table 1).

Treatments	Periods		
	24 H	**48 H**	**72 H**
BBio (mean ± SD)	0.233 ± 0,041 ^{A}	0,145 ± 0,013 ^{A}	0,219 ± 0,010 ^{A}
Biodentine (mean ± SD)	0.088 ± 0.004 ^{B}	0.110 ± 0.007 ^{A}	0.108 ± 0.004 ^{B}
MTA Repair HP (mean ± SD)	0.366 ± 0.029 ^{CD}	0.242 ± 0.007 ^{B}	0.351 ± 0.008 ^{C}
Negative control (C-) (mean ± SD)	0.287 ± 0.016 ^{AC}	0.197 ± 0.011 ^{AB}	0.244 ± 0.012 ^{A}
Positive control (C+) (mean ± SD)	0.378 ± 0.041 ^{D}	0.264 ± 0.019 ^{B}	0.355 ± 0.037 ^{C}
Different superscript letters in the same column indicate statistically significant differences in intergroup comparisons (two-way ANOVA, followed by Tukey test; $p<0.05$).			

Table 1: Cell viability intergroup comparisons.

The intragroup comparisons revealed the statistically significant decreasing of cell viability at 48h in BBio group compared with the other groups. This behavior still occurred with MTA Repair HP and positive Control (C+). Negative control (C-) showed the following cell viability pattern: 24H>72H>48H (Table 2).

	Treatments				
Periods	**BBio** (mean ± SD)	**Biodentine** (mean ± SD)	**MTA Repair HP** (mean ± SD)	**Controle Negativo** (mean ± SD)	**Controle Positivo** (mean ± SD)
24 h	0.233 ± 0.041 ^{A}	0.088 ± 0.004 ^{B}	0.366 ± 0.029 ^{CD}	0.287 ± 0.016 ^{AC}	0.378 ± 0.04 ^{D}
48 h	0.145 ± 0.013 ^{A}	0.110 ± 0.007 ^{A}	0.242 ± 0.007 ^{B}	0.197 ± 0.011 ^{AB}	0.264 ± 0.019 ^{B}
72 h	0.219 ± 0.010 ^{A}	0.108 ± 0.004 ^{B}	0.351 ± 0.008 ^{C}	0.244 ± 0.012 ^{A}	0.355 ± 0.037 ^{C}
Different superscript letters in the same line indicate statistically significant differences in intragroup comparisons (two-way ANOVA, followed by Tukey test; $p<0.05$).					

Table 2: Cell viability intragroup comparisons.

Discussion

For materials intended for use in vital pulp therapy, it is essential to prioritize their interaction with pulp-derived cells. In this context, *in-vitro* cytocompatibility assays serve as an initial and reliable tool to screen materials with regenerative potential. Materials should be capable of maintaining viability and promoting the proliferation of Stem cells from Human Exfoliated Deciduous teeth (SHED), which are believed to play a key role in pulpal repair and healing processes [23]. Therefore, technology and education are mandatory for the evolution of biomembrane compatible with tissue healing and regenerative processes. In this scenario, chitosan, a natural polymer has advantages as biocompatibility, biodegradability, antimicrobial and antifungals properties that benefit dental hard tissues and makes this material a valid, effective and safe option for dental treatments [8].

Tissue bioengineering aims at replacing injured cells and stimulating new tissue growth, to repair the injured one [3]. In this context, chitosan-based pulp capping material is an excellent alternative because the chitosan concentration used to prepare such membranes showed to be compatible, safe and without side effects for human tissues [7,9]. Moreover, this has been studied in bioengineering with positive biocompatible results after contact with human cells [16,17]. Calcium silicate cements have been used due to their low cost, great availability and biocompatibility. Thus, the association of chitosan with calcium silicate cement within a pulp capping membrane (BBio) may have advantages for Dentistry application [8].

Although the literature reports the effects of various bioceramic cements on different cell types, limited data are available regarding their impact on Stem cells from Human Exfoliated Deciduous teeth (SHED). Cell viability studies of other cell types (fibroblast NIH3T3) with chitosan membranes show that either pure chitosan membranes or chitosan membranes associated with other drugs enabled cell growth [9]. This agrees with the cell viability results of this present study, which demonstrated that BBio exhibited cell viability values similar to negative control but higher than Biodentine. On the other hand, MTA Repair HP had higher cell viability values than BBio. We hypothesized that such difference would occur due to the alkaline pH observed in membranes with cements, as pointed out by Lourenço-Neto [8]. Previous studies demonstrate that MTA Repair HP is a material that favors cell viability with high biocompatibility and biomineralization [18], corroborating the results of this present study, in which SHED cell viability after contact with MTA Repair HP was significantly greater than negative control and similar to that of positive control groups. Notwithstanding, Biodentine showed the smallest cell viability values compared with the other materials and non-treated cells, which agrees with recent previous studies [18-20].

Although, *in-vitro* assays using cell cultures are valuable tools for understanding the mechanisms underlying biological responses, they present inherent limitations. The evaluation of biocompatibility should not be restricted to cytotoxicity assays alone. To comprehensively assess a material's regenerative potential, future studies should incorporate additional biological parameters, such as cell migration, proliferation capacity and osteogenic differentiation. It is important to recognize that ideal materials for clinical application must not only be cytocompatible but also exhibit bioactivity-actively promoting cellular responses essential for tissue repair and regeneration [23,24].

Conclusion

Cell viability was influenced by both the type of bioactive material and the exposure time. Although MTA Repair HP presented the best biocompatibility results in the analyzed periods, the biocompatibility of BBio with SHED was similar to the pulp capping materials available in the dental market, proving to be a promising biomaterial.

Conflict of Interest

The authors declare that they have no conflicts of interest with the contents of the article.

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Authors' Contributions

All authors have contributed equally to this work and have reviewed and approved the final manuscript for publication.

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