SHORT COMMUNICATION

O-21. EVALUATION OF THE CYTOTOXICITY OF PULP FLOOR PERFORATION FILLING MATERIALS BY USING IN PARALLEL 2D AND 3D CULTURE MODELS.

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Key words
Dental pulp capping, Toxicity tests, Spheroids, Dental cements.

Introduction

Pulp chamber floor perforation can be a complication of endodontic therapy. To fill the perforations, several materials have been tested (1). Calcium hydroxide-based cements have been used in this goal with inconsistent success for many years (2). Biodentine™ (Septodont, St-Maur-des-Fossés, France) is a new Ca_3SiO_5 material based on Portland cement (3). This material is a dentin substitute, suitable for direct restorative posterior filling for pulp capping and for furcal perforation filling. Multicellular spheroids are an example of an efficient three-dimensional culture model widely used in cancer research. It has been demonstrated that these cell aggregates mimic more accurately the human tissue environment than classic monolayer culture tests (4). The first aim of this study was to investigate the biological effects of Biodentine™ compared with Septocalcine Ultra, a $Ca(OH)_2$ cement, (Septodont, St-Maur-des-Fossés, France) on fibroblast (MRC 5) and osteoblast (Saos-2) cell lines. The second aim was to evaluate its effects on cell viability in a spheroid 3D model compared with a 2D model.

Materials and Methods

Septocalcine Ultra and Biodentine were prepared according to the manufacturer's instructions. The test media were prepared by incubating samples in culture medium and used for direct contact with the two cell lines. The biocompatibility of Biodentine was compared with Septocalcine Ultra using MTT or acid phosphatase viability assays (APH) on monolayer cultures or spheroid cultures on Day 3 and 7. The general features of the spheroids were investigated by SEM. One-way analysis of variance and Fisher Protected Least Significant Difference were used to analyse the resulting data.

Results

The viability of the cells grown with Biodentine was significantly higher compared with Septocalcine in the monolayer model ($p<0.05$). The viability of the MRC 5 spheroids grown with the two test media was significantly higher compared with MRC 5 monolayer cultures ($p<0.05$). The cell-cell junctions of the Saos-2 spheroids looked strongly altered when grown with either of the test media.

Discussion

In the present study, spheroids were used for the first time to assess biomaterials involved in treatment of root canal and floor perforations, or pulp capping. This 3D model reflects

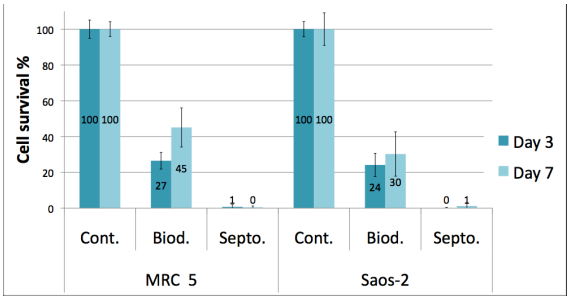

Figure 1: Cytotoxicity of media conditioned with Biodentine and Septocalcine Ultra on MRC 5 and Saos-2 cell lines grown on monolayer culture. Viability was evaluated with the MTT assay after 3 days and 7 days of incubation.

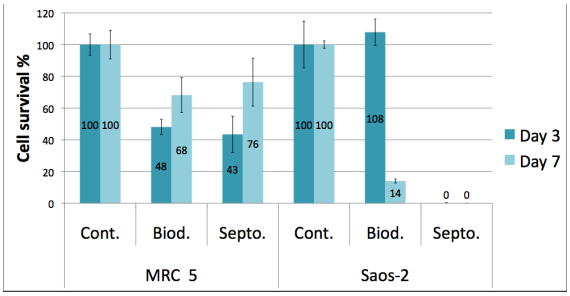

Figure 2: Cytotoxicity of media conditioned with Biodentine and Septocalcine Ultra on MRC 5 and Saos-2 cell lines grown on spheroid culture. Viability was evaluated with the APH assay after 3 days and 7 days

more accurately the clinical expression panels of the tissues than do classic 2D cultures (4).
The results of the MTT assay performed on 2D cultures showed the toxicity of the Septocalcine test medium, which induced the loss of the whole culture after 48 h of contact in both cell lines. This result is not surprising, because the composition of the Septocalcine Ultra is quite similar to Dycal® and the toxicity by direct contact of this material has already been demonstrated (5).
The cell viability of the 2D MRC 5 and Saos-2 cells grown with Biodentine test medium was markedly altered on Day 3 (73% and 76% reduction respectively). Viability reductions had already been observed with mineral trioxide aggregate, another widely researched Ca_3SiO_5 material (6).
The APH test revealed that the MRC 5 cell line grown with Biodentine test medium (BTM) was also prone to a viability reduction when the 3D model was used (52% and 32% of decrease on Days 3 and 7 respectively). More surprisingly, the MRC 5 spheroids grown with Septocalcine test medium (STM) showed similar viability to the spheroids grown with BTM on Days 3 and 7 (43% and 76% of viable cells respectively). The compaction of those spheroids can explain their better resistance compared with the monolayer culture. The existence of a drug concentration gradient inside the spheroids is one of the mechanisms of chemo-resistance responsible for failures of some oncology tested drugs (7).
On Day 3, the viability of the Saos-2 spheroids grown with BTM remained unchanged because, unlike the 2D culture, only the outer cells are really exposed. On the other hand, the viability in 3D culture decreased sharply on Day 7 and became lower than in 2D. This result can be explained by an indirect additional toxicity targeting cell-cell adhesion, which was responsible for the spheroid fragmentation observed with the microscope. The cells died after they detached and allowed the BTM to penetrate more easily in the inner layer of the spheroid. The same issue was observed with the Saos-2 spheroids grown with STM. With this material, the adhesion perturbation was stronger because the spheroid disintegration began from Day 3. The lesser resistance of Saos-2 spheroids to dissociation compared with MRC 5 ones could be explained by the difference of compaction between the spheroids of the two cell lines.
The cytotoxicity of the Septocalcine must be put into perspective because some authors have claimed that pulp capping with $Ca(OH)_2$ liners was an efficient treatment (2). The pulp clearance can make this cytotoxicity not clinically relevant.

Conclusions

The biocompatibility of the Biodentine seemed to be in accordance with its suggested indications. The spheroid model, in contrast to the monolayer model, allowed response differences between cell lines to be shown, and thus seems more suited for assessing the clinical relevance of the materials' cytotoxicity.

Aknowledgements

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