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Pulp fibroblasts and dental materials - an In-vitro-study

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Introduction

Reparative dentinogenesis is the basic mechanism to repair defects after injury or artifical exposition. In fact, pulp fibroblasts have the potential to change into odontoblast like cells in order to produce reparative dentin to conserve the dental pulp. To treat exposed pulps, various restorative dental materials can be used such as calcium hydroxide, cyanoacrylate, mineral trioxide aggregat, adhesives and growth factors but calcium hydroxide is the most important. Since its establishment in the 1920s, one can achieve success in 80 - 90% after direct pulp capping. This success is the result of the cooperation of calcium and hydroxyl ions. While calcium ions have a mitogenic potential to promote migration, differentiation and mineralization, the hydroxyl ions induce a high level of alkalinity to act against inflammation and for the division of cells. But nevertheless, there is a great variety in the tissue reactions after the use of different calcium hydroxide-containing suspensions or cements (1,3).

Objectives

Therefore it was the aim of this study to examine the effect of various calcium hydroxide-preparations on the viability of pulp fibroblasts in cell culture and to compare these with those of other dental materials.

Material and Methods

Human dental pulp cells were obtained from non-carious, freshly extracted third molars and were cultured in D-MEM (PromoCell GmbH, Heidelberg, Germany) containing 10% FCS (SIGMA-ALDRICH-CHEMIE GmbH, Taufkirchen, Germany) and 50 µg/ml Gentamycin (Biochrom AG seromed®, Berlin, Germany).

Assay A:

The first assay was carried out with cells of the third passage in 96well culture plates (10.000 cells/well). Before the beginning of the investigation, the cells were divided in two groups of equal numbers. One group was cultured with 10% FCS and the other with 0.1% FCS only. After two days of adaptation the following materials were applied to the culture well directly:
Dycal® Ivory (Dentsply De Trey, Konstanz, Germany), Calxyl® red (OCO-Präparate, Dirmstein, Germany), zinc oxide-eugenol, a glassionomer (Ketac-Molar® Aplicap®, ESPE, Seefeld, Germany) and the dentin adhesive (OptiBond Solo^{TM}, Kerr Corporation, Orange, California, USA). Further a calcium hydroxide-suspension (0.001137 mg/ml Calxyl® red, pH 8.34) prepared before, was directly supplemented to each well with 1.25 µl (2). The meduim was changed every second day. To measure the viability, the viability test EZ4U (Easy for you, Biozol, Diagnostica Vertriebs GmbH, Eching, Germany) was carried out in this assay after 3, 6, 12 , 24 and 48 hours and 4, 8, 16 and 32 days. The EZ4U test based on the use of tetrazolium salts and the viability is adequate to the extinction measured at 450 nm (reference 620 nm) after four hours of incubation with the EZ4U substrate, wich was given into the culture well directly.

Assay B:

The second assay was carried out with the cells of the fourth passage in 24well culture plates (30.000 cells/well). In comparison to the first assay the cells were not divided in groups and got only 0.1% FCS over the whole period of 32 days. The materials tested were the calcium hydroxide-containing ones of the first assay again: Dycal® Ivory (Dentsply De Trey, Konstanz, Germany), Calxyl® red (OCO-Präparate, Dirmstein, Germany) and the prepared calcium hydroxide suspension (0.001137 mg/ml Calxyl® red), wich was applied to each well with 2.5µl. As indicator for the viability, the EZ4U test was carried out in the same way as described above again after 6, 12, 24 and 48 hours and after 4, 8, 16 and 32 days.

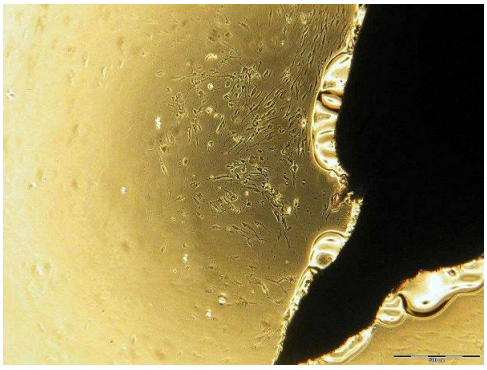

Figure 1: pulp fibroblasts and Dycal&reg after 14 days in culture (assay B)

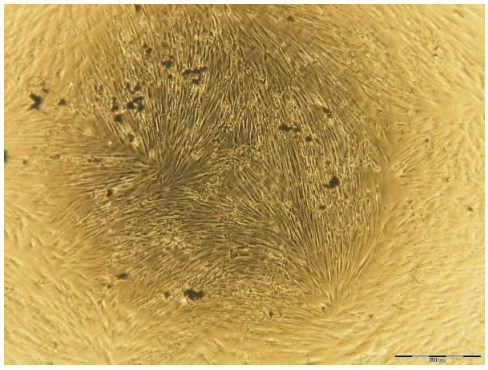

Figure 2: pulp fibroblasts and Calxyl-suspension after 14 days in culture (assay B)

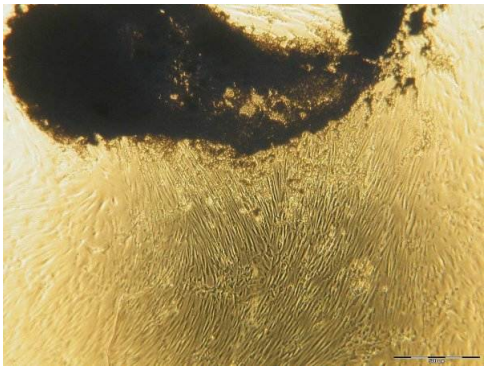

Figure 3: pulp fibroblasts and Calxyl&reg after 14 days in culture (assay B)

Results

Assay A:

Of all materials the pulp fibroblasts showed the lowest decrease in viability after the direct application of the prepared calcium hydroxide-suspension followed by Calxyl® red and Dycal®. The remaining materials: zinc oxide-eugenol, the glassionomer and the dentin adhesive were cytotoxic and reduced the viability of the cells in a short time. But regardless of the material applied, the viability values were nearly always higher, if the cells got the 10% FCS-containing medium.

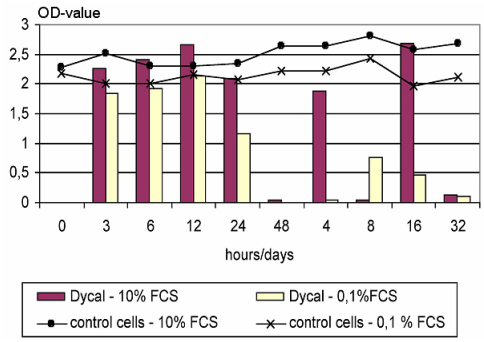

Figure 4a: mean values of the first EZ4U-test (assay A), OD-value - extinction at 450 nm with reference at 620 nm, Dycal®)

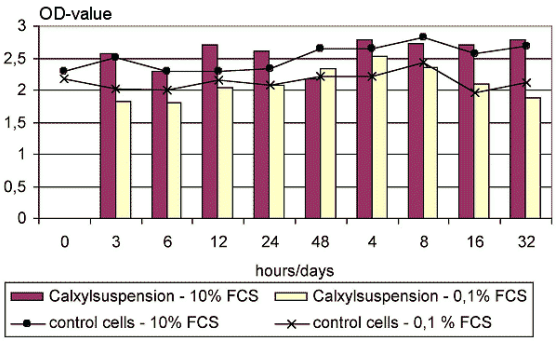

Figure 4b: mean values of the first EZ4U-test (assay A), OD-value - extinction at 450 nm with reference at 620 nm, Calxyl-suspension

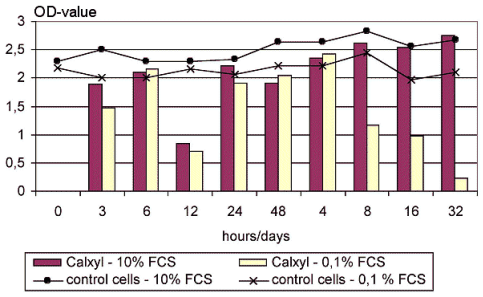

Figure 4c: mean values of the first EZ4U-test (assay A), OD-value - extinction at 450 nm with reference at 620 nm, Calxyl® red

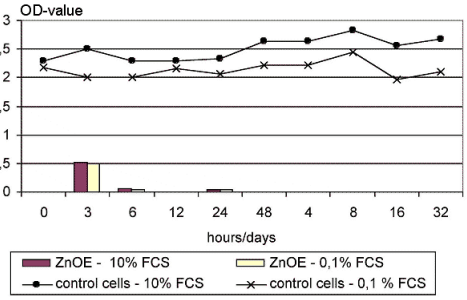

Figure 4d: mean values of the first EZ4U-test (assay A), OD-values - extinction at 450 nm with reference at 620 nm, zinc oxide-eugenol

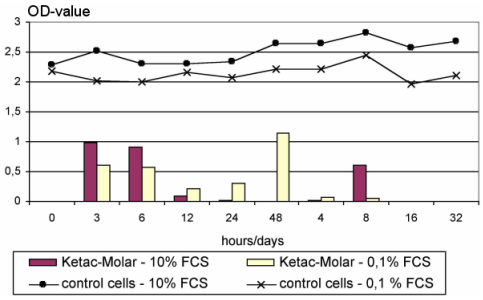

Figure 4e: mean values of the first EZ4U-test (assay A), OD-value - extinction at 450 nm with reference at 620 nm, Ketac-Molar® Aplicap®

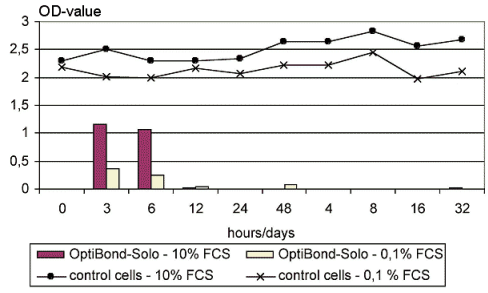

Figure 4f: mean values of the first EZ4U-test (assay A), OD-value - extinction at 450nm with reference at 620 nm, OptiBond Solo™

	Material	Viability of pulp fibroblasts compared with control cells after 32 days	
		D-MEM with 10% FCS	D-MEM with 0.1% FCS
Assay A (96 well)	Calxyl suspension	103.8	89.6
	Calxyl® red	102.5	10.9
	Dycal®	4.7	5.0
	zinc oxide-eugenol	0	0
	Ketac-Molar®	0	0
	OptiBond Solo™	0	0
Assay B (24 well)	Calxyl suspension		149.6
	Calxyl® red		37.9
	Dycal®		0

Table 1: Viability of pulp fibroblasts in comparison to control cells in % after 32 days (the viability of the control cells was determined as 100%)

Assay B:

The result of the second assay confirm the first ones for 0.1% FCS. The calcium hydroxide-suspension and Calxyl® showed the lowest decrease in the viability of the cultured pulp cells again, Dycal® exepted.

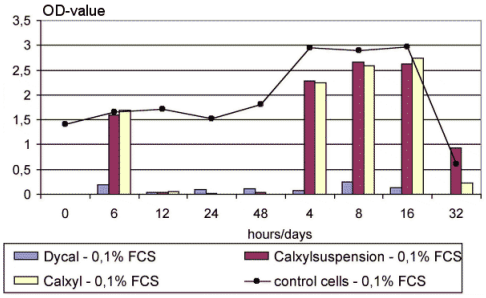

Figure 5: mean values of the second EZ4U-test (assay B), OD-value in extinction at 450 nm with reference at 620 nm

Discussion and Conclusions

The results show that there is a great difference in cell viability based on the materials tested but also on the growth medium used. So, independent of the material applied in the first assay, the viability values (OD-value) were nearly always higher, if the cells got the 10% FCS containing medium. This was probably due to the unknown level of growth factors and other important nutrient supplements in the serum. But nevertheless only these cells were viable over the whole peroid, wich got the calcium hydroxide-containing materials. The other materials were cytotoxic, because their application led to a rapid decrease in viability after a short time. As described above, there are different tissue reactions to different calcium hydroxide-containing materials. So in the first assay the calcium hydroxide-suspension will show the lowest decrease in viability followed by Calxyl® and Dycal®. To check the results of the calcium hydroxide-containing materials again, a second assay was carried out. Here we used 24well culture plates, because in this way a greater number of cells would get more place to react with the material applied. The cells also got only the 0.1% FCS containing medium to take away the influence of highly concentrated growth factors and other supplements. At the end of the study the results were comparable to those of the first assay. So, the calcium hydroxide-suspension treated pulp cells showed the lowest decrease in viability again followed by Calxy®, but with the exeption of Dycal®.
It was concluded that the direct application of aqueous calcium hydroxide-suspension was the best to save the viability of the cultured human pulp fibroblasts in this study.

Bibliography

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2. Torneck, C.D.: Moe, H.; Howley, T.P.: The feffect of calcium hydroxide on porcine pulp fibroblasts in vitro. J Endodont 9, 131 (1983)
3. Tziafas, D.: Economides, N.: Formation of crystals on the surface of calcium hydroxide-containing matreials in vitro. J Endodont 25, 539 (1999)

Abbreviations

- FCS - Fetal calf serum
- D-MEM - Dulbeccos modified Eagles Medium

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Poster Faksimile:

Pulp fibroblasts and dental materials – an In-vitro-Study

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Introduction and Objectives:

Reparative dentinogenesis is the basic mechanism to repair dentin defects after injury or artificial exposition. In fact, pulp fibroblasts have the potential to change into odontoblast like cells in order to produce reparative dentine to conserve the dental pulp. To treat exposed pulps, various dental materials can be used such as calcium hydroxide, cyanoacrylate, mineral trioxide aggregate, adhesives and growth factors, but calcium hydroxide is the most important. Since its establishment in the 1920´s, one can achieve success in 80-90% after direct pulp capping today. This success is the result of the cooperation of calcium and hydroxyl ions. While calcium ions have a mitogenic potential to promote migration, differentiation and mineralization, the hydroxyl ions induce a high level of alkalinity to act against inflammation and for division of cells . But nevertheless, there is a great variety in the tissue reactions after the use of different calcium hydroxide containing suspensions or cements [1, 3].
Therefore, it was the aim of the study to examine the effect of various calcium hydroxide preparations on the viability of pulp fibroblasts in cell culture and to compare these with those of other dental materials.

Material and Method:

Human dental pulp cells were obtained from non-carious, freshly extracted third molars and were cultured in D-MEM (Dulbecco´s modified Eagles Medium, PromoCell GmbH, Heidelberg, Germany) containing 10% FCS (Fetal calf serum, SIGMA-ALDRICH-CHEMIE GmbH, Taufkirchen, Germany) and 50 µg/ml Gentamycin (Biochrom AG seromed®, Berlin, Germany).

Assay A:
The first assay was carried out with cells of the third passage in 96well culture plates (10.000 cells/well). Before the beginning of the investigation, the cells were divided in two groups of equal numbers. One group was cultured with 10% FCS and the other with 0.1% FCS only. After two days of adaptation the following materials were applied to the culture well directly:
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Assay B:
The second assay was carried out with the cells of the fourth passage in 24well culture plates (30.000 cells/well). In comparison to the first assay, the cells were not divided in groups and got only 0.1% FCS over the whole period of 32 days. The materials tested were the calcium hydroxide containing ones of the first assay again: Dycal® Ivory (Dentsply De Trey, Konstanz, Germany), Calxyl® red (OCO-Präparate, Dirmstein, Germany) and the prepared calcium hydroxide solution (0.001137 mg/ml Calxyl® red), which was applied to each well with 2.5 µl. As indicator for the viability, the EZ4U test was carried out in the same way as described above again after 24 and 48 hours and 4, 8, 16 and 32 days.


Figure 1: pulp fibroblasts and Dycal® after 14 days in culture (assay B)


Figure 2: pulp fibroblasts and Calxyl suspension after 14 days in culture (assay B)


Figure 3: pulp fibroblasts and Calxyl® red after 14 days in culture (assay B)

Results:

Assay A:
Of all materials the pulp fibroblasts showed the lowest decrease in viability after the direct application of the prepared calcium hydroxide suspension followed by Calxyl® red and Dycal®. The remaining materials: zinc oxide-eugenol, the glassionomer and the dentine adhesive were cytotoxic and reduced the viability of the cells in a short time. The viability values were nearly always higher, if the cells got the 10% FCS-containing medium.

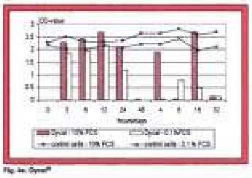
Fig. 4a: Dycal®

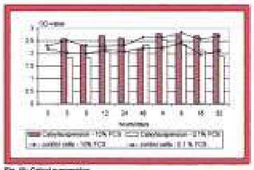
Fig. 4b: Calxyl suspension

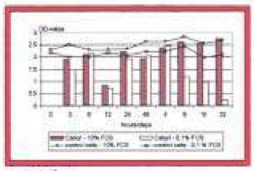
Fig. 4c: Calxyl® red

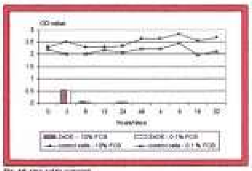
Fig. 4d: zinc oxide-eugenol

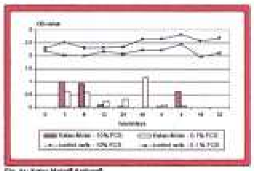
Fig. 4e: Ketac-Molar® Aplicap®

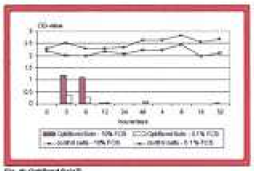
Fig. 4f: OptiBond Solo™

Figure 4a-f: mean values of the first EZ4U-test (assay A), OD-value = extinction at 450nm with reference at 620nm

Table 1: Viability of pulp fibroblasts in comparison to control cells in % after 32 days (the viability of the control cells was determined as 100%)

		[illegible]	
		[illegible]	[illegible]
[illegible]	Calxyl suspension	[illegible]	[illegible]
	Calxyl® red	[illegible]	[illegible]
	Dycal®	[illegible]	[illegible]
	zinc oxide-eugenol	0	0
	Ketac-Molar®	0	0
	OptiBond Solo™	0	0
[illegible]	Calxyl suspension		[illegible]
	Calxyl® red		[illegible]
	Dycal®		0

Assay B:
The results of the second assay confirm the first ones for 0.1% FCS. The calcium hydroxide suspension and Calxyl® red showed the lowest decrease in the viability of the pulp cells again ,Dycal® excepted.

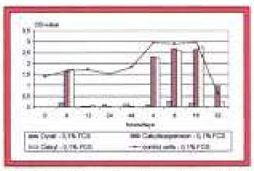
Figure 5: mean values of the second EZ4U-test (assay B), OD-value = extinction at 450nm with reference at 620nm

Discussion and Conclusions:

The results show that there is a great difference in cell viability based on the materials tested but also on the growth medium used. So, independent of the material applied in the first assay, the viability values (OD-values) were nearly always higher, if the cells got the 10% FCS containing medium. This was probably due to the unknown level of growth factors and other important nutrient supplements in the serum. But nevertheless only these cells were viable over the whole period, which got the calcium hydroxide containing materials. The other materials were cytotoxic, because their application led to a rapid decrease in viability after a short time. As described above , there are different tissue reactions to different calcium hydroxide-containing materials. So, in the first assay the calcium hydroxide suspension will show the lowest decrease in viability followed by Calxyl® and Dycal®. To check the results of the calcium hydroxide containing materials, a second assay was carried out. Here we used 24 well culture plates, because in this way a greater number of cells would get more place to react with the material applied. The cells also got only the 0.1% FCS containing medium to take away the influence of highly concentrated growth factors and other supplements. At the end of the study, the results were comparable to those of the first assay. So, the calcium hydroxide suspension showed the lowest decrease in viability again followed by Calxyl®, but with the exception of Dycal®. It was concluded that the direct application of aqueous calcium hydroxide suspensions will show the lowest decrease in viability of human pulp fibroblasts.