Nanohydroxyapatite Used as a Pulpotomy and Direct Pulp Capping Agent in Primary Pig Teeth

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ABSTRACT

Purpose: Recently, a fully Nanocrystalline Hydroxyapatie (**NHA**) paste has been introduced for augmentation procedures in osseous defects and is attracting increasing interest in medicine and dentistry. The purpose of the present study was to assess and compare the pulp response of pig primary teeth after capping with NHA and formocresol in pulpotomy and NHA and calcium hydroxide in direct pulp capping.
Methods: Forty teeth of two 4-month old pigs were pulpotomized and capped with these materials. Four weeks later, the animals were euthanized and the specimens were prepared for histological examination.
Results: In the pulpotomy groups, there was a significant difference between NHA and FC in terms of pulp response, hard tissue formation and normal pulp tissue preservation. In the direct pulp capping groups, there was no significant difference between NHA and Ca(OH)2 in terms of criteria mentioned above.
Conclusions: The results of the present histological study show that, in the short term and in non-carious pig teeth, NHA appears to be biocompatible and provokes no moderate or severe inflammatory reaction in pulp tissue in both pulpotomy and direct pulp capping treatments.
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Dental decay in primary teeth remains a considerable health problem. Where decay extends to involve the dental pulp, pulp treatment techniques are often used to manage both symptomatic and asymptomatic teeth.

Pulpotomy is a procedure in which the infected coronal pulp is amputated and the vital surface of radicular pulp tissue is treated with a medicament or dressing material to promote healing in the underlying tissue. For half a century, formocresol (**FC**) is the most commonly used medicament in pulpotomy procedures for primary teeth. Formocresol has 2 major components: (1) formaldehyde; and (2) cresol. The formaldehyde interacts with the protein portion of cells and is responsible for the fixation process, and the cresol reduces the irritation caused by the formaldehyde and increases its germicidal effect.[1,2] The well-known toxic and carcinogenic potential of formaldehyde,[3,5] however, has today become a concern in pediatric dentistry.

Direct pulp capping (**DPC**) is one of the most controversial treatments for primary teeth. This treatment consists of dressing exposed pulp to maintain pulp vitality throughout the life of the tooth. In primary teeth, however, the use of DPC is more limited than in

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permanent teeth because, compared to permanent teeth, primary teeth are smaller overall, have less enamel and dentin coverage and have relatively larger pulp chambers and pulp horns. This means that pulp infection can occur earlier in primary teeth as a result of a more rapid progression of caries and thinner hard tissues.

DPC is traditionally performed with various formulations of calcium hydroxide—**$Ca(OH)_2$**—which helps to produce dentinal bridges on the exposed pulpal area and whose high pH has a bactericidal effect. The major disadvantage of $Ca(OH)_2$ is that it dissolves over time. Since most dentin bridges form under the $Ca(OH)_2$, they may contain tunnels and allow pulp to become infected or necrotic due to microleakage.[6] Recent research into pulp treatment materials has focused on what is called "biological era" materials.[7] These biomaterials encourage dentin and bone regeneration/formation.

Mineral trioxide aggregate (**MTA**) is the most well-documented new material to appear in the last decade. MTA is composed primarily of tricalcium and dicalcium silicate and has been recommended for pulp capping,[8,9] perforation sealing,[10] and apexification[11] in permanent teeth. In the field of pediatric dentistry, human studies have reported that MTA was biocompatible and could preserve pulpal health as well as promote healing and pulp regeneration.[12,13]

Hydroxyapatite (**HA**) has already been used in bone grafts in orthopedic,[14] dental, and maxillofacial applications[15-17] due to its chemical and structural similarity to bone and teeth. Despite its biocompatibility, one of the problems related to HA is the release of crystals or agglomerates that could impair cell activity and hinder the regeneration processes. As natural bone has nanoscale features, it is believed that nanostructured HA could improve the properties of synthetic bone due to its higher surface area.

Recently, a fully synthetic nanocrystalline hydroxyapatite (**NHA**) paste containing approximately 65% water and 35% nanostructured apatite particles was introduced for augmentation procedures in cases of osseous defects.[18] Compared to bulk materials, the advantages of such a nanostructured material are its close contact with surrounding tissue, its rapid resorption capacities, and the high number of molecules on its surface. The NHA paste is attracting increasing interest in medicine and dentistry and has already been used in orthopedic surgery[19] and for jaw cysts[20] and periimplantitis lesions.[21] Theoretically, the biocompatibility of NHA, combined with its structural similarity to teeth, may allow NHA to stimulate odontoblasts, thus promoting the formation of dentin bridges.

To our knowledge, no previous animal or human study has investigated the interaction between NHA and primary teeth pulp tissue in the case of pulpotomy and DPC. Consequently, the aim of the present study was to assess and compare the pulp response of primary pig teeth after capping with nanocrystalline hydroxyapatite and formocresol in pulpotomy and NHA and calcium hydroxide in direct pulp capping.

METHODS

All experimental procedures were approved by the Animal Ethics Commission of the Medical Sciences Division at the Free University of Brussels, Brussels, Belgium. Forty primary teeth from 2 healthy 4-month-old female pigs were used in this study—8 incisors and 12 molars per pig. All 4 canines were used as an untreated control group. After an injection of 10 mg/kg Ketamine HCl (Ketamine 1000 CEVA, Ceva Santé Animale, Libourne, France) and 2 mg/kg xylazine hydrochloride (Rompun, Bayer, Kiel, Germany), all teeth were subjected to cavity preparation under general anesthesia. An infiltration of mepivacaine hydrochloride 3% (Scandonest

Table 1. Scores Used During the Histological Exams

Criterion	Score
Inflammatory cell response	0=none of a few scattered inflammatory cells beneath the pulp exposure site
	1=mild inflammatory cells, such as mono- or polymorphonuclear leukocytes
	2=moderate inflammatory cell infiltration involving the third coronal radicular pulp
	3=severe inflammatory cell infiltration involving the third coronal or more radicular pulp
Tissue disorganization	0=normal tissue beneath the pulp exposure site
	1=odontoblast-like cells, odontoblasts, and pulp tissue pattern disorganization or odontoblast hyperactivity, but normal central pulp tissue pattern
	2=general disorganization of the pulp tissue pattern
	3=pulp necrosis
Hard tissue formation	0=no hard tissue formation
	1=incomplete hard tissue formation
	2=thick hard tissue formation

Table 2. Grading of Histological Features for Each Material Based on the Scores Given in Table 1

Procedure	Materials	Inflammatory cell response				Pulp tissue disorganization				Hard tissue formation		
		0	1	2	3	0	1	2	3	0	1	2
Pulpotomy	Nanocrystalline hydroxyapatite (NHA)	9*	1*	0	0	7†	3†	0	0	0	1	9‡
Direct pulp capping	Formocresol (FC)	1*	8*	1	0	1†	8†	1	0	10‡	0	0
	NHA	9	1	0	0	8	2	0	0	0	0	10
	Calcium hydroxide $Ca(OH)_2$	9	1	0	0	7	3	0	0	0	1	9

** Indicates a significant difference between the NHA group and the FC group in terms of inflammatory cell response (P>.001).*
† Indicates a significant difference between the NHA group and the FC group in terms of pulp tissue disorganization (P>.001).
‡ Indicates a significant difference between the NHA group and the FC group in terms of hard tissue formation (P>.01).

3%, Septodont, Saint-Maur, France) was used as a local anesthesia. Due to difficulties in applying a rubber dam to pig teeth, it was not used in this experiment. The teeth were kept dry using gauze swabs and then wiped with chlorhexidine digluconas 0.2% solution (Corsodyl, Glaxo-SmithKline, Genval, Belgium) for 1 minute.

PULPOTOMY GROUPS

After exposing the pulp chamber using a high-speed carbide bur, the coronal pulp was removed with a round bur. Bleeding was controlled with sterile cotton pellets. The teeth of each hemimaxillary segment (4 incisors and 6 molars) were used for one comparison. The maxillary and mandibular right teeth of the first pig were assigned to the formocresol group, and the maxillary and mandibular left teeth were assigned to the NHA group.

In the FC group, the radicular pulp stumps were covered for 5 minutes with a cotton pellet moistened with 20% formocresol (Rockle's 4, Septodont, Saint-Maur, France) that is similar to the 1:5 dilution of Buckley's formocresol solution.

In the NHA group, the radicular stumps were covered with NHA paste (Ostim, Heraeus Kulzer, GmbH, Hanau, Germany).

DPC GROUPS

All teeth were subjected to a Class V preparation on their buccal surfaces. Bleeding was controlled with sterile cotton pellets, and, as in the pulpotomy groups, the teeth of each hemimaxillary segment (4 incisors and 6 molars) were used for one comparison. The maxillary and mandibular right teeth of the second pig were assigned to the $Ca(OH)_2$ group, and the maxillary and mandibular left teeth were assigned to the NHA group.

In the $Ca(OH)_2$ group, Dycal (Dentsply DeTrey GmbH, Konstanz, Germany) was mixed according to the manufacturer's instructions and applied over the exposed tissue.

In the NHA group, the exposed pulp was covered with NHA paste (Ostim, Heraeus Kulzer, GmbH, Hanau, Germany).

Following these procedures, the coronal cavities of each tooth in both groups were filled with IRM (Dentsply DeTrey GmbH).

During experimental period, the 2 female pigs, both from the same litter, were kept in individual cages in the medical research animal care unit of the Free University of Brussels. After 4 weeks, the animals were euthanized by administering embutramide 4 to 6 ml/50 kg (T61, Intervet Int, Unterschleißheim, Germany). Following the euthanasia, both jaws were removed from each pig, fixed in a 10% neutral-buffered formalin solution, and decalcified in Surgipath Decalcifier I (Surgipath, Grayslake, Ill). The jaw segments were then prepared for histological examination—they were embedded in paraffin and sectioned to a thickness of 6 µm.

All sections were viewed under a light microscope and were evaluated according to the criteria listed in Table 1 by 2 examiners who were not informed about the aim of study and the materials used. Interexaminer agreement was satisfactory. The 8 control canines allowed us to make a histological comparison of the treated and nontreated teeth.

The data collected from the histological examinations (Table 2) were statistically analyzed using Mann-Whitney and Dunn multiple comparison tests. The statistical analyses were performed using Prism software v 3.0 (GraphPad Software, San Diego, Calif), with the significance level set at $P<.05$.

RESULTS

In the pulpotomy groups, there was a significant difference between NHA and FC in terms of pulp response ($P>.001$), hard tissue formation ($P>.001$), and normal pulp tissue preservation ($P>.01$). There was, however,

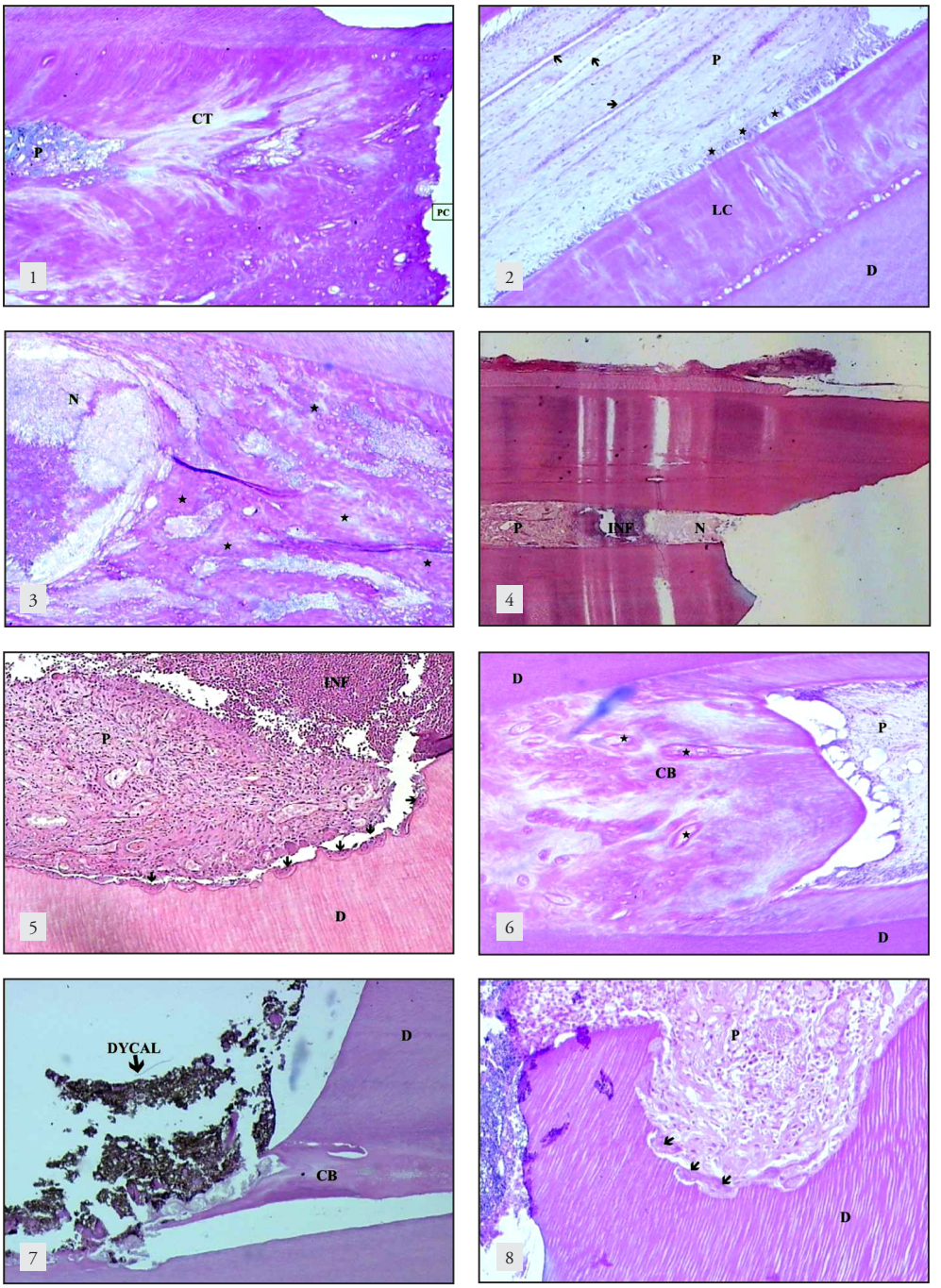

Figure 1. Pulpotomy with NHA (Ostim), maxillary second molar (H&Ex4). CT: calcified tissue, P: pulp, PC: pulp chamber.

Figure 2. Pulpotomy with NHA (Ostim), mandibular central incisor (H&Ex4). Notice a thick lateral calcification (LC), hyperactivity of odontoblastic layer (★) and enlarged blood vessels (➔) in pulp tissue (P). D: dentin.

Figure 3. Pulpotomy with NHA (Ostim), maxillary lateral incisor (H&Ex4). Calcified tissue (★) formation under superficial necrotic tissue (N).

Figure 4. Pulpotomy with FC (Rockle's 4), maxillary central incisor (H&Ex 2.5). The most common pattern of pulp tissue in FC group, necrotic layer (N), acute inflammation (INF) and a normal pulp tissue (P).

Figure 5. Pulpotomy with FC (Rockle's 4), mandibular third molar (H&Ex4). Internal resorption at the cervical third of root canal. ↓: giant cells, P: pulp, D: dentin, acute inflammation (INF).

Figure 6. Direct pulp capping with NHA (Ostim), maxillary first molar, (H&Ex4). Notice a normal pulp tissue (P) under a thick calcified bridge (CB). ★: osteoid dentin, D: dentin.

Figure 7. Direct pulp capping with calcium hydroxide (Dycal), mandibular central incisor (H&Ex4). Calcified bridge: CB, D: dentin.

Figure 8. Direct pulp capping with calcium hydroxide (Dycal), mandibular first molar (H&Ex4). Internal resorption (↙) D: dentin, P: pulp.

no significant difference between incisors and molars in each group in terms of the aforementioned criteria. In the DPC groups, there was no significant difference between NHA and $Ca(OH)_2$ in terms of pulp response, hard tissue formation, and normal pulp tissue preservation, nor was there a difference between incisors and molars in each group in terms of aforementioned criteria.

PULPOTOMY

NHA. Nine samples displayed a calcified bridge under the pulpotomy site (Figure 1). Two samples presented lateral cal-cification (Figure 2). One sample showed necrotic tissue just under pulpotomy site, with a calcified bridge being observed under the necrotic tissue (Figure 3). This necrotic tissue could be related to blood clot formation.

FC. Eight samples showed an acute inflammation under necrotic tissue, but the pulp architecture under the inflammation was normal (Figure 4). One sample showed an inflammatory reaction in the middle of the pulp canal. One sample presented normal pulp tissue. Three samples showed lateral calcification, and 2 samples showed internal resorption (Figure 5).

DPC

NHA. All samples showed a calcified bridge at the pulp exposure site (Figure 6). Eight of the specimens presented a normal histological pulp pattern and odontoblast layer. One specimen showed a mild inflammation beneath the pulp exposure.

$Ca(OH)_2$. Nine samples displayed a complete calcified bridge under the exposure site (Figure 7). One sample presented an incomplete calcified bridge with an acute inflammatory reaction under the exposure site. Two samples showed internal resorption (Figure 8).

DISCUSSION

Hydroxyapatite is widely used to repair, fill, extend, and reconstruct damaged bone tissue. Natural bone is composed of calcium phosphate with nanometric needle-like crystals of approximately 5 to 20 nm. Calcium phosphate ceramics have been used in dentistry and orthopedics for over 30 years because of its similarity to the mineral phase of natural bone, if abstraction is made of its constituent particle size. Nanophase calcium phosphates can mimic the dimensions of the constituent components of natural tissues and modulate enhanced osteoblast adhesion and resorption with long-term functionality of tissue-engineered implants. Several researchers have tried to customize its properties—bioactivity, mechanical strength, and solubility—by controlling its composition, morphology, and nanoparticle size.[22,23] Thus, nanotechnology can play an important role in the development of porous bioceramics with high mechanical strength, as well as enhanced bioactivity and resorbability.[24] Using nanotechnology, calcium and phosphate can be manipulated at the molecular level and assembled to produce materials with unique structural and functional properties.

NHA was used in the present study as a pulp dressing material for primary teeth because it is highly biocompatible and encourages hard tissue regeneration. The results of this study confirm NHA's biocompatibility. No signs of moderate or severe pulp inflammatory response, though one sample in both the pulpotomy and DPC groups displayed a localized layer of mild inflammation just beneath the exposure site. Our study also showed that NHA promoted a hard tissue formation in the pulp of primary pig teeth. Cells situated on the NHA material tend to exhibit high motility because of their well-developed philopodia and lamellopodia.[25] Thus, NHA may also serve as a chemoattractant for developing cell philopodia because of selective absorption of specific proteins. Since collagen enhances the ability of osteoblasts to attach, NHA would facilitate osteoblast migration, or in our study, odontoblast-like cell migration.

For this study's DPC groups, we did not use any bacterial staining procedure. It is generally accepted that the microscopic evaluation of tooth tissue sections using the modified gram staining procedure is not a very reliable method for detecting micro-organisms. This is because histological processes (decalcification) could eliminate bacteria or reduce the effectiveness of bacterial staining procedures, especially in pig's teeth, because decalcification may take a long time.[26]

During bone regeneration after injury, the inflammatory phase is followed by a reparative phase, characterized by the formation of a hard callus that occurs when the matrix mineralizes. Within 3 to 6 weeks, the new bone acquires a trabecular pattern, which may be observed histologically.[27] As the processes of bone and tooth mineralization are similar, we set the experimental period at 4 weeks to determine the potential for a reparative phase following the inflammatory phase for each material. We found that NHA material had a much greater potential for reparation following inflammation in pulpotomy treatment.

We used a local anesthetic with animals that had general anesthesia because the trauma of surgery, with the associated tissue damage, sensitizes the peripheral nervous system. The barrage of nociceptive input produces sensitization of the neurons of the dorsal horn of the spinal cord. Pain, however, should not be considered as just electrical activity elicited by peripheral stimulation and/or tissue damage. As local and regional anesthetic techniques are the only ones which produce a complete block of the peripheral nociceptive input, they are the most effective means of preventing sensitization of the central nervous system and the development of pain.[28] It has been reported that supplementing a general anesthetic

protocol by the addition of local anesthesia has significant analgesic benefits and less supplemental anesthetic is required.[29]

CONCLUSIONS

The results of the present histological study showed that, in the short term and in noncarious pig teeth, nanocrystalline hydroxyapatite appears to be biocompatible and provoke no moderate or severe inflammatory reaction in pulp tissue in both pulpotomy and direct pulp capping treatments. Nevertheless, these results were obtained for healthy pulp tissue, and the correlation with the response in inflamed pulp should be made with caution. Clearly, the presence of bacteria or their metabolites could provoke pulp degeneration and expressive molecular alterations. Therefore, additional studies are needed to investigate the response of these agents to the pulp tissue that has previously been injured during the caries process. In addition, further research with larger samples and a more extended study time is necessary. Clinical studies also should be encouraged. Despite the need for further study, based on our results, it is fair to say that biological compounds and new materials (eg, mineral trioxide aggregate, beta-tricalcium phosphate,[30] and NHA) may completely transform our philosophy of pulp treatment in the future.

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