

1 **Microcrystalline hydroxyapatite is not inferior to fluorides in clinical caries**
2 **prevention: a randomized, double-blind, non-inferiority trial**

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35 **Abstract**

36 Recent evidence for a significant interference of microcrystalline hydroxapatite (HAP)
37 particles with re- and demineralisation processes at the tooth-biofilm interface sug-
38 gested, that they may be promising candidates for efficacious caries prevention.
39 This multicenter randomized controlled non-inferiority trial evaluated the impact of the
40 2 x daily use of a HAP dentifrice without fluoride on the progression of enamel caries
41 in adolescent caries-risk patients subjected to orthodontic therapy, with a fluoridated
42 AmF/SnF dentifrice serving as a positive control. Primary study outcome was the
43 occurrence of enamel caries lesions \geq ICDAS (*International Caries Detection and*
44 *Assessment System*) code 1 around orthodontic brackets on the vestibular surfaces
45 of teeth 15-25 within the 168 days observation period. Secondary study outcomes
46 were the occurrence of enamel caries lesion \geq ICDAS code 2, Plaque Index (PII) and
47 Gingival Index (GI). Out of 150 recruited patients, 147 were included in the intent to
48 treat analysis (ITT); 133 finished the study per protocol (PP). PP data analysis
49 revealed the occurrence of enamel caries \geq ICDAS code 1 in 54.7% of the HAP
50 group patients compared to 60.9% of the fluoride control. In the ITT analysis the
51 corresponding numbers were 56.8% (HAP) and 61.6% (control). Non-inferiority
52 testing of the ITT as well as the PP data set proved that the caries preventive efficacy
53 of the HAP dentifrice was not inferior to the protection provided by the fluoridated
54 AmF/SnF control. Regarding all assessed secondary outcomes (enamel caries \geq
55 ICDAS code 2, GI, PII) no significant differences between both experimental groups
56 were observed. Within the restraints set by design and study population of this trial
57 microcrystalline HAP as ingredient of toothpaste may thus be regarded a promising
58 supplement to fluorides in clinical caries prevention (ClinicalTrials.gov:
59 NCT02705456).

60 Introduction

61 In recent years findings, mostly derived from *in vitro* studies, suggested, that micro-
62 crystalline hydroxyapatite (HAP) particles may be promising candidates for the
63 prevention of cariogenic demineralization and the stimulation of remineralization
64 processes on enamel and dentine surfaces [1-3]. Huang et al. (2011) reported a
65 regain of mineral content and an increase in microhardness on demineralized bovine
66 enamel slabs that had subsequently been exposed to microcrystalline HAP particles
67 [2]. The observed increase in mineral content proved to be pH-dependent and was
68 significantly higher under acidic conditions. Lin et al. (2014) discovered a significant
69 inhibition of future demineralisation under acidic conditions after HAP application due
70 to the formation of a protective HAP layer over the prism-prism sheath interfaces,
71 where enamel dissolution usually is initiated [4]. In an *in situ* - study the use of a zinc-
72 carbonate HAP microcluster-containing mouth rinse significantly reduced bacterial
73 colonization on bovine enamel slabs worn intraorally by healthy volunteers [5].
74 Hannig and Hannig (2010) put these *in situ* and *in vitro* findings into a more compre-
75 hensive perspective by stating that already physiological tooth wear constantly
76 releases HAP particles into the oral environment, which may subsequently interfere
77 with de- and remineralisation processes as well as with the metabolism of the oral
78 microbiota at the tooth-bacterial biofilm interface [6]. The impact of microcrystalline
79 HAP as an ingredient of dentifrices has been positively evaluated in controlled clinical
80 trials regarding dentinal hypersensitivity [7-10] and parameters of periodontal health
81 [11]. Up to date, however, comparable data regarding the caries-preventive
82 properties of HAP toothpastes are mostly missing. They are limited to positive
83 findings from *in situ* studies on extracted teeth or standardized enamel and dentine
84 specimen, being subjected to different toothpaste slurries and worn in between by

85 volunteers in intraoral appliances [12-15]. As orthodontic therapy with fixed
86 appliances is known to be associated with an increased incidence of the overgrowth
87 of a caries-promoting microbiota [16] and the development of white spot enamel
88 caries lesions [17-19], this study aimed at the assessment of the caries-preventive
89 impact of the regular use of a fluoride-free HAP dentifrice in this particular group of
90 caries risk patients. Due to the abundant evidence for the caries preventive efficacy
91 of fluorides [20, 21], clinical caries studies may no longer involve a true negative
92 control for obvious ethical reasons. Thus a non-inferiority trial was conducted. The
93 study hypothesis to be tested was, that, in terms of caries prevention, the regular use
94 of the HAP test dentifrice is not inferior to the regular use of a fluoridated control with
95 proven efficacy.

96

97 **Material and methods**

98 The investigation was designed as a multicenter, prospective, parallel group, two
99 arm, double-blind, randomized clinical non-inferiority trial to be performed at the
100 German study centers Wuerzburg (leading study center), Regensburg, Munich,
101 Dresden and Frankfurt. The study protocol was prepared in accordance with the
102 declaration of Helsinki and met the criteria of GCP. It was approved by the ethics
103 committee of the University of Wuerzburg (file #184/13) on March 28th, 2013.
104 Registering clinical trials was not yet generally regarded a mandatory prerequisite to
105 be performed prior to study initiation in 2012 and 2013 during the planning phase of
106 this investigation. Therefore, registration with ClinicalTrials.gov (identifier:
107 NCT02705456) was performed late on February 25th, 2016 in the final phase of
108 patient recruitment, which had started already more than 1 year earlier. The authors
109 confirm that all ongoing and related trials for this drug/intervention are registered.

110 **Study design**

111 The design of the study is schematically depicted in Fig 1.

112

113 **Fig 1. Study Design**

114

115 At visit 1 (-4 to -28 days prior to baseline) patients scheduled for orthodontic therapy
116 were screened for study eligibility. Those meeting it and giving informed consent
117 were subsequently scheduled for the baseline visit 2 (day 0).

118 At visit 2 Plaque Index (PII) as well as Gingival Index (GI) scores were recorded from
119 the vestibular surfaces of teeth 15 to 25 followed by professional tooth cleaning and
120 the subsequent assessment of the vestibular enamel surfaces of teeth 15 to 25
121 according to ICDAS II criteria. Afterwards orthodontic brackets were adhesively
122 mounted to the vestibular surfaces and any excess of adhesive resin was removed.
123 No sealants, fluoride varnishes or any other caries-preventive layers surrounding the
124 brackets were applied. Using a randomization list a supply of either the test dentifrice
125 or the control dentifrice, calculated to be adequate for 4 weeks of 2 x daily repeated
126 toothbrushing, as well as a standardized electric tooth brush (Oral-B Pulsar 35;
127 Procter & Gamble GmbH, Germany) to be used for the duration of the study, were
128 handed over to the study patients. The dosage of the assigned dentifrice (2 x daily a
129 streak of approx. 1 g) and the use of the electric tooth brush were practically
130 instructed and the patients informed, to bring back all assigned toothpaste tubes at
131 the next scheduled visit. At day 28 the sequence of recording PII, GI and ICDAS II
132 scores was repeated as described for visit 2. As an additional caries-preventive
133 measure, teeth 15-25 were disinfected with a topically applied 1% chlorhexidine
134 (CHX) gel. Toothpaste tubes supplied at visit 2 were taken back and a new supply for
135 the next 4 weeks handed over. At day 56 (visit 4) oral hygiene was reinstructed and

136 the cleaning/disinfection procedures as well as the return/handing over of the
137 toothpaste supply performed as described before. At day 84 (visit 5) the recording of
138 GI, PII and ICDAS II scores as well as cleaning and disinfection were repeated as
139 described before. Next to a new supply of toothpaste also a new Pulsar 35 electric
140 toothbrush was handed over. At day 112 (visit 6) and at day 140 (visit 7) procedures
141 performed were identical to those at day 56 (visit 4). At day 168 (visit 8) the final
142 assessment of PII, GI and ICDAS II scores as well as the return of the study
143 dentifrices was conducted as described before. Furthermore, at each study visit
144 patients were asked about the occurrence of important harms or unintended effects
145 related or unrelated to the use of the study dentifrices.

146

147 **Study population**

148 The trial was performed in adolescents and young adults being scheduled for
149 orthodontic therapy with fixed appliances.

150

151 *Inclusion criteria were:*

152 - age 11-25 yrs.

153 - scheduled orthodontic therapy with fixed appliances of at least 6 months duration

154 comprising the placement of orthodontic brackets on the vestibular surfaces of

155 teeth 15 - 25

156 - regular (2x daily) oral home care with toothbrush and toothpaste

157 - caries promoting salivary counts of mutans streptococci $\geq 10^5$ CFU/ml, which were

158 determined using the CRT[®] bacteria test (Ivoclar Vivadent, Liechtenstein) [22].

159

160

161 *Exclusion criteria were:*

162 - untreated caries lesions of ICDAS code 3-6 on any tooth

163 - treated carious lesions of ICDAS code 3-6 on the vestibular surfaces

164 of teeth 15-25

165 - diseases or conditions interfering with the salivary flow or requiring the regular use

166 of medications interfering with it

167 - antibiotic therapy within the last 6 weeks before study participation or necessity for

168 antibiotic prophylaxis during dental interventions

169 - known allergies to ingredients of the experimental dentifrices

170

171 **Interventions - experimental dentifrices**

172 *a) Test Dentifrice*

173 The test dentifrice (Karex[®] Zahnpasta; Dr. Kurt Wolff GmbH & Co. KG, Germany)

174 was provided by the sponsor of the study. It contained 10% of microcrystalline HAP

175 as the main caries-preventive agent and also the following ingredients: Aqua,

176 glycerol, hydrogenated starch hydrolysate, xylitol, hydrated silica, Silica, aroma,

177 cellulose gum, sodium methyl cocoyl taurate, *Helianthus annuus* seed oil, polyglyceryl-

178 3 palmitate, polyglyceryl-6 caprylate, *Usnea barbata* extract.

179

180 *b) Control Dentifrice*

181 A commercially available fluoridated toothpaste (meridol[®] Zahnpasta; CP GABA

182 GmbH, Germany) was used as a positive control. It contained amine fluoride and

183 stannous fluoride in concentrations of 350 ppm and 1050 ppm, respectively, and

184 furthermore the following ingredients: Aqua, sorbitol, hydrated silica, silica dimethyl

185 silylate, hydroxyethylcellulose, PEG-40, hydrogenated castor oil, cocamidopropyl

186 betaine, aroma, sodium gluconate, PEG-3 tallow aminopropylamine, saccharin,
187 hydrochloric acid, potassium hydroxide, CI 74160.

188

189 **Primary outcome**

190 The primary outcome was set to the percentage of subjects in each experimental
191 group exhibiting the new occurrence of at least one enamel caries lesion of ICDAS
192 code 2 or higher on any vestibular surface of the 10 evaluated teeth 15 to 25 during
193 the observation period of 168 days.

194 **Assessment of carious lesions**

195 The occurrence of caries lesions was evaluated visually on the vestibular surfaces of
196 teeth 15 to 25 according to the criteria of the *International Caries Detection and*
197 *Assessment System* (ICDAS-II) [23]. The examination was performed at baseline,
198 prior to the fixation of the orthodontic brackets, and was repeated 28 days, 84 days
199 and 168 days later. All teeth were professionally cleaned before each assessment
200 from any adhering bacterial biofilms or stains. The development of a caries lesion
201 > ICDAS code 3 during the course of the study on any tooth and observed at any visit
202 was defined as an immediate study exit criterion.

203 **Interexaminer reliability**

204 To ensure interexaminer reliability, prior to the study onset all examiners were
205 instructed to pass the ICDAS e-learning course at the icdas.org website and were
206 subsequently trained in person by an experienced expert (K.H.K.) to perform ICDAS
207 assessments in reference patients. Grading skills were retrained 3 times during the
208 course of the study using another internet-based ICDAS training tool. Similar to Luz
209 et al. [24] it confronted the examiners with a random sample of 40 pictures of upper

210 premolars, canines and incisors with surface integrities ICDAS codes 0-3. 50% of
211 the pictures of a given sample were randomly presented in duplicates to evaluate the
212 ability of the examiners to reproduce their own assessments.
213 Interrater reliability analysis revealed a mean weighted kappa = 0.75 for the first
214 assessment run, which increased to kappa = 0.80 for the final calibration, indicating
215 "substantial agreement" among the different examiners throughout the study [25].
216 Although up to three examiners were trained and calibrated at each study center
217 before the onset of the trial, at four centers the bulk of the practical evaluations was
218 performed by a single principal examiner (Munich 100% of all visits, Frankfurt 100%,
219 Regensburg 96 %, Wuerzburg 96 %) At the center in Dresden the principal examiner
220 performed 58% of all examinations, the second examiner 38%.

221

222 **Secondary outcomes**

223 Secondary outcomes were plaque coverage and gingival inflammation at baseline
224 and at day 168 evaluated by recording the
225 - Plaque Index (PII) [26] and the
226 - Gingival Index (GI) [27], respectively.

227

228 **Sample size calculation**

229 Based on a reported caries incidence rate of about 60% in a preceding caries trial
230 assessing orthodontic patients with fixed braces, who were not being preselected
231 for particular caries-promoting risk factors [18], the likelihood for the occurrence of an
232 ICDAS code 2 lesion during the 168 day observation period in this cohort of caries-
233 risk individuals with elevated salivary numbers of caries-promoting mutans
234 streptococci was extrapolated to be p=80% for the control group using the fluoridated

235 toothpaste. The difference between both experimental groups not be regarded
236 clinically relevant was set to $\Delta \leq 20\%$. A sample size of 2 x 74 study patients was
237 calculated to be sufficient to reject the null hypothesis, that the test dentifrice is
238 inferior to the control dentifrice, using a non-inferiority margin of $\Delta = 20\%$ for the
239 primary outcome measure and one-sided, exact Fisher Test ($\alpha = 5\%$, power = 80%).
240

241 **Blinding, randomisation**

242 The trial was designed to blind study patients and examiners to the group assign-
243 ment. For this purpose, both study dentifrices (test/control) were filled into neutral
244 plastic tubes of identical shape and color by an independent, GMP certified
245 laboratory for cosmetics. Using block randomization with a block size of 4 a random
246 list was generated to code-label test and control tubes with consecutive unique
247 identification numbers. Randomization of dentifrice assignment was stratified by
248 study center. Handing out of the experimental dentifrices to the study patients
249 followed the sequence of the identification numbers and was performed by trained
250 study nurses not involved in the examination of the study participants. To maintain
251 blinding of examiners and study patients, the study patients were instructed not to
252 discuss toothpaste-related issues with the examiners but with the study nurses only,
253 who were also responsible for instructing the patients in efficacious oral hygiene and
254 taking back the empty or unused dentifrice tubes at the subsequent visits. The
255 number of study nurses varied between a minimum of one and a maximum of four
256 per study center.
257

258 **Statistical analysis**

259 The primary outcome measure was analysed primarily for the PP population and
260 repeated for sensitivity reasons, for the ITT population. The exact confidence limits
261 (Clopper-Pearson) were computed to test non-inferiority (cp. [28]). For the primary
262 outcome measure, non-inferiority was claimed, if the upper limit of the one-sided 95%
263 confidence for the corresponding difference between test and control dentifrice was
264 less than $\Delta \leq 20\%$.

265 In addition, two-sided Wilcoxon-Mann-Whitney tests were used for between group
266 comparisons and Friedman tests for within group comparisons for secondary
267 outcomes.

268 SAS[®] 9.3 software package (SAS Institute Inc., USA) was used for statistical
269 evaluations.

270

271 **Patient Recruitment**

272 Out of a total of 281 screened individuals, 150 patients meeting the inclusion criteria
273 gave written informed consent and were recruited at the study centers in Wuerzburg
274 (n=36), Regensburg (n=72), Dresden (n=28), Munich (n=12) and Frankfurt (n=2). The
275 first patient was included in the trial on November 13th, 2013, the last patient left the
276 trial on August 28th, 2016. At the study centers Wuerzburg, Regensburg and Dresden
277 not only center patients but also orthodontic patients being treated in private practice
278 were included and assessed by the examiners of the center.

279

280

281

282

283 **Dropouts**

284 Six patients of the test group and 4 patients of the control group terminated study
285 participation prematurely due to lack of interest or not keeping the follow-up appoint-
286 ments. Further 6 patients completed the study but were excluded from the PP
287 analysis due to insufficient dosing of the assigned dentifrice, calculated from the
288 residual weight of the returned dentifrice tubes. All but one patient of the test group
289 and all patients of the control group received at least one dose of the assigned
290 dentifrice (n=149) and were thus primarily included in the ITT analysis set. As two
291 study patients left the trial already before the first reevaluation at week 4, the total
292 number of study individuals suitable for an inclusion in the ITT analysis of caries
293 development further decreased to n=147. No important harms or unintended effects
294 related or unrelated to the use of the study dentifrices were reported. Finally, a total
295 of 133 study patients (64 test / 69 control) was included in the PP analysis set (Fig 2).

296

297 **Fig 2. CONSORT Flow Chart**

298

299 **Results**

300 *Health Status, Age, Gender*

301 All study patients were healthy. Mean age of the HAP test group patients was 13.4
302 yrs \pm 1.8 SD and 13.4 yrs \pm 1.7 SD for the fluoride control group. 52.7% of the HAP
303 test group and 62.2% of the fluoride control group patients were female.

304

305 *Blinded change of the primary outcome*

306 A blinded analysis of the ICDAS data at the end of the study revealed, that the
307 overall observed occurrence of ICDAS lesions \geq code 2 in the study population was
308 29.3% and therefore considerably lower than the anticipated value ($p = 80\%$) used
309 for the sample size calculation. As the difference between the groups not be
310 regarded clinically relevant had been set in the study protocol to $\Delta \leq 20\%$ a clinically
311 meaningful verification of non-inferiority was no longer warranted. Thus, the primary
312 endpoint was changed to the more frequent overall occurrence of ICDAS lesions
313 \geq code 1 (59.2%). It was decided to keep the original primary endpoint as an
314 additional secondary outcome in the statistical data analysis.

315

316 *Occurrence of ICDAS lesions \geq code 1 and \geq code 2*

317 The occurrence of ICDAS lesions \geq code 1 (revised primary outcome) and ICDAS
318 lesions \geq code 2 (secondary outcome) is depicted in table 1. In the PP analysis
319 54.7% of the HAP group patients ($n=35$) and 60.9% of the fluoride control group
320 patients ($n=42$) showed the formation of at least one ICDAS lesion \geq code 1 during
321 the 168 day observation period. In the ITT analysis the corresponding numbers were
322 56.8% for the patients of the HAP group ($n=42$) and 61.9% for those of the fluoride
323 control ($n=45$). In the PP data set the occurrence of at least one ICDAS lesion \geq code
324 2 was observed in 23.4% of the patients of the HAP group compared to 34.8% of the
325 fluoride controls. In the ITT data set the the corresponding numbers were 25.7% of
326 the HAP group and 32.9% of the fluoride controls. Differences between the groups
327 could not be verified statistically for both analysis sets.

328

329

330

331 **Table 1. Occurrence of ICDAS lesions \geq code 1 and \geq code 2 within the**
 332 **168 days observation period (ITT and PP analysis)**

333

Treatment Group	ICDAS lesion	per protocol (PP) analysis			intent to treat (ITT) analysis		
		%	n	N	%	n	N
HAP test	ICDAS code \geq 1 ^a	54.7	35	64	56.8	42	74
AmF/SnF control	ICDAS code \geq 1 ^a	60.9	42	69	61.6	45	73
HAP test	ICDAS code \geq 2	23.4	15	64	25.7	19	74
AmF/SnF control	ICDAS code \geq 2	34.8	24	69	32.9	24	73

334 ^a primary outcome measure; n, number of patients with ICDAS lesions \geq code 1 and \geq code 2;
 335 N, number of patients in corresponding treatment group

336

337

338 *Non-inferiority analysis*

339 Table 2 displays the difference between both experimental groups regarding the
 340 percentage of study subjects experiencing the new occurrence of at least one ICDAS
 341 lesion \geq code 1 (primary outcome) or at least one ICDAS lesion \geq code 2 (secondary
 342 outcome) including the corresponding one-sided 95% confidence intervals. As the
 343 upper limits of the 95% confidence intervals for the primary outcome are well below
 344 the given non-inferiority margin of $\Delta \leq 20\%$ for both analysis sets (PP: 8%; ITT: 9%)
 345 the HAP group has to be considered as non-inferior to the fluoride control.
 346 Also regarding the secondary outcome (ICDAS lesion \geq code 2) the upper limits of
 347 the 95% confidence intervals are substantially below the given non-inferiority margin
 348 of 20% for both analysis sets (PP: 3%, ITT: 7%), indicating that again the HAP test
 349 group has to be considered being non-inferior to the fluoridated control.

350

351

352

353 **Table 2. Difference between both experimental groups regarding the**
 354 **occurrence of ICDAS lesions \geq code 1 and \geq code 2 within the 168 days**
 355 **observation period (95% one-sided confidence intervals)**

Analysis	ICDAS lesion	Proportion in risk difference	Exact lower one-sided 95% confidence limit	Exact upper one-sided 95% confidence limit ^b
PP analysis	ICDAS code \geq 1 ^a	-0.062	-0.203	0.083
ITT analysis	ICDAS code \geq 1 ^a	-0.048	-0.188	0.087
PP analysis	ICDAS code \geq 2	-0.114	-0.255	0.030
ITT analysis	ICDAS code \geq 2	-0.072	-0.202	0.068

357 ^a primary outcome measure; ^b the upper one-sided 95% confidence limit is markedly lower than the
 358 non-inferiority margin of 0.20 ($\Delta = 20\%$) thus inferiority is rejected.

359
 360 *Effect of study site on the primary outcome measure*

361 The effect of study site on the primary outcome measure Δ ICDAS score \geq 1 at week
 362 24 was evaluated by logistic regression analysis. It included the factors study site,
 363 treatment group and the interaction between study site and treatment group. Due to
 364 small sample sizes, the data for the study sites Dresden, Munich and Frankfurt were
 365 pooled (n=40 patients). The results revealed a significantly lower incidence of the
 366 primary outcome at week 24 ($p < 0.001$) at the combined smaller centers (Dresden,
 367 Munich, Frankfurt) when compared to the study centers in Regensburg (n=72
 368 patients) or Wuerzburg (n=35 patients). However, there was no significant interaction
 369 between study site and treatment group, proving that the factor study site did not
 370 significantly affect efficacy differences between the treatment groups (for further
 371 informations see also Appendix 1).

372
 373 *Number and severity of ICDAS score increases*

374
 375 The number and severity of ICDAS score increases on the vestibular surfaces of
 376 teeth 15-25 over the course of the study are shown in table 3. At week 4 3.2% of the
 377 teeth in the HAP group were already affected (ICDAS code 1: 3.0%; ICDAS code 2:

378 0.2%) compared to 3.6% of the AmF/SnF control group (ICDAS code 1: 3.1%;
379 ICDAS code 2: 0.5%). These figures steadily increased over time. At week 24
380 19.6% of the teeth in the HAP group were affected (ICDAS code 1: 14.8%; ICDAS
381 code 2: 4.8%) compared to 21.0% in the AmF/SnF control group (ICDAS code 1:
382 14.2%; ICDAS code 2: 6.7%; ICDAS code 3: 0.1%).
383
384

385 **Table 3. Number and severity of ICDAS score increases observed on teeth 15-**
 386 **25 at week 4, week 12 and week 24 (PP data set; n=133)**
 387

		HAP test group		AmF/SnF control group		Total	
Visit	Δ ICDAS	n teeth	%	n teeth	%	n teeth	%
week 4	No increase	620	96.9	665	96.4	1285	96.6
	Δ ICDAS code 1	19	3.0	24	3.5	43	3.2
	Δ ICDAS code 2	1	0.2	1	0.1	2	0.2
	Total	640	100.0	690	100.0	1330	100.0
week 12	No increase	573	89.5	611	88.6	1184	89.0
	Δ ICDAS code 1	58	9.1	59	8.6	117	8.8
	Δ ICDAS code 2	9	1.4	20	2.9	29	2.2
	Total	640	100.0	690	100.0	1330	100.0
week 24	No increase	514	80.3	545	79.0	1059	79.6
	Δ ICDAS code 1	95	14.8	98	14.2	193	14.5
	Δ ICDAS code 2	31	4.8	46	6.7	77	5.8
	Δ ICDAS code 3	0	0	1	0.1	1	0.1
	Total	640	100.0	690	100.0	1330	100.0

388

389

390 *Plaque Index (PII), Gingival Index (GI)*

391 The results of the ITT analysis of the PII and the GI data are shown in Table 4. Mean
 392 PII as well as mean GI scores increased significantly ($p < 0.0001$) between baseline
 393 and day 168 in both groups. Neither at baseline nor at day 168 differences between
 394 the experimental groups were statistically significant.

395

396

397

398 **Table 4. Plaque Index and Gingival Index scores at baseline, day 28, day 84 and**
 399 **day 168 (ITT analysis)**
 400

Plaque Index (PII)						
	HAP test group			AmF/SnF control group		
Visit	N	mean ^a	SD	N	mean ^a	SD
baseline ^b	75	0.35	0.37	74	0.36	0.36
week 4	74	0.65	0.58	72	0.76	0.56
week 12	74	0.72	0.60	73	0.75	0.61
week 24 ^b	74	0.85	0.66	73	0.77	0.61
Gingival Index (GI)						
	HAP test group			AmF/SnF control group		
Visit	N	mean ^a	SD	N	mean ^a	SD
baseline ^b	75	0.29	0.36	74	0.37	0.41
week 4	74	0.53	0.57	73	0.58	0.54
week 12	74	0.51	0.53	73	0.66	0.55
week 24 ^b	74	0.70	0.56	73	0.77	0.59

401
 402 ^a significant ($p < 0.0001$) increase of Plaque Index and Gingival Index over time from baseline to day
 403 168 for both treatment groups (Friedman test)

404 ^b no significant differences between both treatment groups at baseline and at day 168
 405 (two-sided Wilcoxon-Mann-Whitney test)
 406

407 **Discussion**

408 **Methods**

409 Caries detection and grading in this trial followed the principles of ICDAS-II [23], an
410 internationally established, state of the art caries assessment method, which is
411 particularly suitable and appropriate for the differentiation and grading of incipient
412 enamel caries development, allowing to verify even minor differences in the efficacy
413 of caries-preventive measures. As described before, repeated examiner calibration
414 by an internet-based ICDAS training tools as well as personal examiner calibrations
415 by an experienced ICDAS grading expert (K.H.K.) were integral parts of the study
416 design, to warrant the validity of the clinical recordings. Mean weighted kappa for
417 interrater reliability increased from 0.75 for the first to 0.80 for the final calibration
418 assessment. This was overall in the upper range of kappa reliability scores reported
419 by other controlled clinical trials and indicated "substantial" agreement [25]. It
420 assured that the assessment of the primary study outcome was based on a sound
421 foundation. Furthermore, all examiners were blinded to the dentifrice allocation of the
422 study subjects throughout the course of the study to avoid any possible examiner
423 bias.

424

425 **Study population**

426 The assessed study patients, wearing fixed orthodontic appliances, were without
427 doubt caries-active, documented by the considerable increase in enamel caries
428 lesions during the 168 days observation period, which was comparable in its
429 magnitude to observations made by other clinical trials [18, 29]. Due to the inevitable
430 lack of a negative control group for ethical reasons, it is however very difficult to
431 assess the true extent of caries prevention provided by the regular use of both

432 dentifrices to the study participants. It may be argued, that in the chosen setting of
433 caries-active orthodontic patients overly acidic conditions beyond the remineralizing
434 capacities of fluorides and hydroxyapatite particles might have rendered a
435 meaningful non-inferiority analysis impossible. We cannot share, however, this
436 assumption for the following reasons: In a more recent multicenter caries trial by
437 Sonesson et al. (2014), assessing a comparable cohort of 424 adolescent patients
438 age 12-16 subjected to orthodontic therapy with fixed appliances, the regular use of a
439 highly concentrated 5000 ppm fluoride dentifrice was accompanied by a significantly
440 lower incidence of white spot enamel lesions when compared to the regular use of a
441 standard 1450 ppm fluoride control dentifrice [30]. While this suggests, that 1450
442 ppm may not be the optimal fluoride concentration for a dentifrice to be used in
443 caries-active orthodontic patients, it evidently contradicts the assumption, that in
444 these patients the caries-preventive efficacy of fluoride is completely blocked by
445 overly acidic conditions. On the contrary, Sonesson et al. even speculated, that the
446 reduced increase in enamel caries observed for the use of the 5000 ppm fluoride
447 dentifrice may be attributable to a dose-dependent inhibitory effect of fluorides on *in*
448 *vivo* lactate production in supragingival bacterial biofilms as discovered by Takahasi
449 and Washio (2012) [31]. The findings by Sonesson et al. are also in line with the
450 conclusions of two meta-reviews assessing the efficacy of topical fluorides in
451 orthodontic patients subjected to therapy with fixed braces, which advocated the
452 additional use of fluoride rinses or varnishes as a complement to regular
453 toothbrushing with fluoride dentifrices [17, 32]. From a clinician's point of view the
454 caries protection provided by the sole use of both dentifrices evaluated in the present
455 trial may not have been sufficient for a sizeable part of the study participants. This
456 may however not be interpreted as an inherent and complete lack of clinical efficacy.
457 It rather reflects the fact, that the study population deliberately and in accordance

458 with the recommendations of the 2004 International Consensus Workshop on caries
459 clinical trials [33], included many subjects with a very high caries activity. While this is
460 a condition, which clinically may only be partially controllable by the sole use of
461 conventional fluoride toothpaste not exceeding the legal fluoride concentrations limits
462 for cosmetic products, as described before, the inclusion of highly caries-active
463 individuals in a controlled caries prevention trial is an indispensable prerequisite for a
464 meaningful verification of possible differences regarding the caries-protecting
465 capacities of evaluated products or interventions [33] .

466

467 Data Analysis

468 Whether the occurrence frequency of ICDAS code 1 enamel caries lesions used in
469 this study is the most suitable primary endpoint for a non-inferiority caries trial may
470 be subject to discussion. However, the adjunctive analysis of the PP data set
471 regarding frequency and severity of the occurrence of enamel caries lesions during
472 the observation period depicted in table 3 only confirms the identified absence of
473 relevant differences between both experimental groups. It may also have been
474 debatable to keep the original non-inferiority margin of $\Delta = 20\%$ when switching the
475 primary outcome of the trial despite an overall incidence of the revised primary
476 outcome (ICDAS lesion code 1) of only 60%. The subsequent analysis of the
477 unblinded PP data set however revealed, that the actual difference between both
478 experimental groups was 6.2% in favour of the HAP test dentifrice with an exact
479 upper one-sided 95% confidence limit of 8.3%, i.e. substantially lower than the preset
480 non-inferiority margin of $\Delta = 20\%$.

481 Secondary Outcomes

482 The data for the secondary outcomes Plaque Index (PII) and Gingival Index (GI)
483 furthermore confirmed the findings of preceding studies, reporting a significant

484 increase of gingival inflammation and bacterial plaque mass after the onset of
485 orthodontic therapy with fixed appliances [18, 29]. Differences between both
486 experimental groups regarding the recorded PII and GI data could not be verified
487 statistically for any of the evaluated time points, which is also in good agreement with
488 the results of a previous trial comparing the plaque- and gingivitis-reducing properties
489 of a fluoride-free HAP test dentifrice and a fluoridated AmF/SnF control in a study
490 cohort of periodontitis patients [11].

491 Outlook

492 While the safety of fluoride-based caries prevention has been firmly established by
493 numerous studies [21], dosage and toxicity aspects have always to be considered.
494 This particularly limits the clinical feasibility of the aforementioned increase in fluoride
495 dosing in high caries-risk infants and children up to an age of 8 due to the
496 associated risk for the development of dental fluorosis. Although not verified by
497 clinical studies so far, increasing the dosing or application frequency of HAP
498 toothpaste might also have a beneficial impact on clinical outcome in highly caries
499 active subjects because HAP is a potent buffer, able to neutralize organic acids in a
500 dose-dependent manner. By contrast to fluorides, increasing the applied dosage of
501 HAP particles is virtually free of any toxicity issues even in infants and children, as
502 HAP is the major mineral phase of all hard tissues within the human body [6].

503

504 **Conclusions**

505 Regular toothbrushing with a fluoride-free microcrystalline hydroxyapatite-containing
506 dentifrice is a viable method of clinical caries control. The proof of non-inferiority in
507 comparison to the regular use of a conventional fluoride dentifrice verified by this
508 trial in a cohort of caries-active adolescents and adults may however be only a first
509 step. The promising results revealed by the present study need to be corroborated by

510 subsequent clinical investigations in a broader spectrum of study populations and
511 diverging caries activities before general conclusions regarding the benefits and
512 limits of microcrystalline HAP in clinical caries prevention may be possible.

513

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Screening Visit 1 (Day -4 – -28)

Baseline Visit 2 (Day 0)
ICDAS, GI, PII
Fixation of Orthodontic Brackets
Randomized handing over of experimental dentifrice

**hydroxyapatite
test dentifrice**

Visit 3 (Day 28)
ICDAS, GI, PII
CHX-disinfection
supply of dentifrice

Visit 4 (Day 56)
CHX-disinfection
supply of dentifrice

Visit 5 (Day 84)
ICDAS, GI, PII
CHX-disinfection
supply of dentifrice

Visit 6 (Day 122)
CHX-disinfection
supply of dentifrice

Visit 7 (Day 140)
CHX-disinfection
supply of dentifrice

**fluoridated AmF/SnF
control dentifrice**

Visit 3 (Day 28)
ICDAS, GI, PII
CHX-disinfection
supply of dentifrice

Visit 4 (Day 56)
CHX-disinfection
supply of dentifrice

Visit 5 (Day 84)
ICDAS, GI, PII
CHX-disinfection
supply of dentifrice

Visit 6 (Day 122)
CHX-disinfection
supply of dentifrice

Visit 7 (Day 140)
CHX-disinfection
supply of dentifrice

Visit 8 Final Visit (Day 168)
ICDAS, GI, PII
Return of assigned dentifrice



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	6-7
	4b	Settings and locations where the data were collected	11
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5-6
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8-9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	12
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	10
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	10
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	10
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	10
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	10

		assessing outcomes) and how	
Statistical methods	11b	If relevant, description of the similarity of interventions	5-6
	12a	Statistical methods used to compare groups for primary and secondary outcomes	10
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	11
	13b	For each group, losses and exclusions after randomisation, together with reasons	11
Recruitment	14a	Dates defining the periods of recruitment and follow-up	11
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	12
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	11
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	13-15
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	-
Harms	19	All important in each group (for specific guidance see CONSORT for harms)	11
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	15-16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	15-16
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	7

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.