

SUPPLEMENTARY MATERIAL

Limeback H, Enax, J, Meyer F. Biomimetic hydroxyapatite and caries prevention: a systematic review and meta-analysis. *Can J Dent Hyg.* 2021;55(3):148–59.

Table S4. Summary of in vivo/in situ studies of HAP-containing oral products with anticaries properties

Study author (country)	Subjects	HAP product	Controls	Study design and length	Experimental conditions	Blinding?	Examiner calibration?	% difference	p value	Conclusions	Comments on the quality of the study
Sudrajat et al. 2020 <sup>41</sup> (Indonesia)	34 children (mean age 8.9 years)	HAP gel	Calcium levels compared to the baseline	In vivo trial to measure calcium deposits in dental biofilm 3 days	Gel applied professionally 3X/day Energy-dispersive X-ray spectroscopy to measure calcium and phosphorus in the collected dental biofilm	NR*	NR	Ca: 60% increase in median % weight P: 20% increase in median % weight	NR	Calcium and phosphorus levels in dental biofilm increased after exposure to the HAP gel (tendency; not statistically significant)	Biofilm calcium and phosphate were measured (this is an important anticaries mechanism) Subjects were children
Wierichs et al. 2020 <sup>42</sup> (Germany)	650 tooth blocks in 20 subjects (ages 20 years to 56 years)	Biorepair® (fluoride-free, zinc carbonate HAP)	Lavera (fluoride-free) Crest® (1100 ppm fluoride) Colgate® Duraphat® (5000 ppm fluoride)	In situ randomized crossover trial on remineralization of tooth surfaces 250 bovine enamel and 400 demineralized dentin blocks mounted on mandibular appliances 4 weeks, 22 h/day exposure Biofilm-covered slabs immersed 40 min, 3X/day, dipped in 10% sucrose	Extraoral exposure to sugar and no hygiene of the appliances Application of test paste was by expectoration of paste slurry onto the appliance Analysed demineralization and remineralization of blocks with transverse microradiography	Double blinded	NR	HAP showed signs of demineralization The fluoride-positive controls showed remineralization	0.028 0.012	This in situ model failed to show non-inferiority to fluoride toothpaste in promoting remineralization of dentin and enamel	Study failed to show significant effect on enamel and dentin de- and remineralization compared to the fluoride positive controls
Nobre et al. 2020 <sup>43</sup> (Germany)	192 blocks in 2 subjects (age 30 years and 35 years)	Three different 5% HAP solutions (particles ranged from 40 nm to 200 nm in size, needle and spherical)	Pure water negative control	In situ trial on HAP attachment to enamel 96 blocks per subject to examine HAP attachment to pellicle-covered blocks 2-hour exposures	Blocks of bovine enamel, titanium, ceramic, and polymethyl methacrylate resin on intraoral splints 30 sec. HAP rinse, up to 2 hours in situ Blocks analysed by SEM, TEM	NR	NR	NA	NA	No quantitative measurement but described nature of HAP particle attachment to intraoral surfaces including enamel	Qualitative examination of HAP attachment to enamel
Nobre et al. 2020 <sup>44</sup> (Germany)	40 blocks in 5 subjects (ages 18 years to 35 years)	Kalident (5% HAP solution)	0.2% chlorhexidine in 7% alcohol or dH2O	In situ crossover trial on biofilm reduction 8 titanium blocks per subject 2-hour exposures	30 sec. HAP rinse, then up to 2 hours in situ Fluorescent microscopy, TEM, SEM, EDX analysis and BacLight viability test	NR	NR	HAP reduced biofilm on polished titanium by 55% and non-polished titanium by 26% Did not significantly affect viability scores	<0.05 <0.05 0.93 0.17	Pure HAP significantly reduced biofilm formation on titanium, similar to chlorhexidine/alcohol rinse	There was quantitative HAP interference of biofilm growth on titanium without being cytotoxic to the bacteria
Bossù et al. 2019 <sup>45</sup> (Egypt)	81 children (ages 7 years to 10 years)	Biorepair® with HAP	1. Neutro-pasta (no active ingredient) 2. Fluoride toothpaste (500 ppm) 3. Fluoride toothpaste (1400 ppm)	In vivo trial on surface effects and biofilm levels Primary tooth blocks from extracted teeth 15-day exposure to pastes before extraction	7- to 10-year-old donors of extracted teeth following treatment (before orthodontics) VP-SEM analysis Surface roughness tested Bacteria levels measured with crystal violet	NR	NR	Significantly smoother enamel surface (indicating repair) compared to the negative water and positive control (fluoride) Nearly 100% reduction in <i>S. mutans</i> CFU	<0.001 <0.001	The HAP toothpaste protects deciduous enamel in the same manner as fluoride toothpastes and inhibits <i>S. mutans</i>	Compared HAP protective effects on primary teeth to fluoride toothpaste HAP = fluoride Novel in vivo design (exposure then in vitro analysis) Subjects were children
Amaechi et al. 2019 <sup>46</sup> (Germany)	30 adults (ages 18 years to 60 years)	Kinder Karex with 10% HAP	Elmex (500 ppm fluoride)	In situ crossover, 2-arm trial on demin/remin Primary tooth blocks 14 days per arm	30 adults wearing appliances Microradiography to monitor remineralization of primary tooth blocks	Double blinded	NR	Both pastes had >50% remineralization and >25% reduction of lesion depth	<0.0001	Kinder Karex with HAP worked as well as 500 ppm fluoride toothpaste to prevent demineralization and help remineralize primary tooth enamel	Compared HAP effects on demin/remin to fluoride toothpaste HAP = fluoride

Table S4. continued

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Al Asmari & Almutairi 2019 <sup>47</sup> (Saudi Arabia)	50 subjects (ages 30 years to 60 years) 25 test, 25 control	Biorepair® mouthwash	0.1% chlorhexidine mouthwash	RCT on biofilm reduction No mechanical plaque control for 2 weeks, then rinse 2x/day with mouthwash another 2 weeks	Plaque index, gingival index	Double blinded	Kappa = 0.88	76% reduction in biofilm compared to baseline	0.001	Biorepair is an effective antiplaque mouthwash superior to 0.1% chlorhexidine	Well-conducted clinical trial HAP is better than 0.1% chlorhexidine in reducing biofilm accumulation
Hagenfeld et al. 2019 <sup>48</sup> (Germany)	41 mild to moderate periodontitis subjects (ages 18 years to 75 years)	Biorepair® (Zn substituted carbonate hydroxyapatite)	Meridol® (Amine fluoride/stannous fluoride)	RCT on biofilm and gingivitis Routine brushing during the 12-week periodontal treatment trial	Random biofilm sampling and 16s RNA sequence identification	Single blinded	NA	No difference	>0.1	No difference between HAP toothpaste and antimicrobial AmF/SnF <sub>2</sub> toothpastes The tested antibacterial and anti-adhesive ingredients have similar impact on the dental microbiome during periodontal therapy	A standard clinical trial on plaque index with sufficient power to detect difference between HAP and a fluoride toothpaste HAP = fluoride
Kensche et al. 2017 <sup>49</sup> (Germany)	36 slabs in 9 volunteers (ages 23 years to 45 years)	5% HAP solution	Meridol® (0.2% chlorhexidine-digluconate)	In situ trial on HAP adhesion to enamel Maxillary splints with 4 bovine enamel blocks 1 min. rinsing, then 8-hour intraoral exposure	DAPI staining for bacterial adhesion BaLight assay for viability SEM, TEM	NR	NR	Nearly 100% reduction in bacterial adhesion (comparable to CHX) No noticeable effect on <i>S. mutans</i> viability	<0.001	HAP has significant bacterial anti-adhesive effects	A multifaceted in situ study on bacterial adhesion HAP reduced bacterial adhesion without being cytotoxic
Kensche et al. 2016 <sup>50</sup> (Germany)	48 enamel slabs in 8 volunteers (ages 24 years to 42 years)	Biorepair® (1% HAP mouthwash)	Tooth Mousse (10% CPP-ACP) Elmex Kariesschutz (150 ppm NaF, 100 ppm amino fluoride)	In situ trial to measure protection from erosion	6 bovine enamel slabs per maxillary splint Exposure intraorally for 28 min. to mouthwash or cream 2 min. HCl incubation Ca release measured with Arsenazo III; phosphate measured with malachite green TEM	NR	NR	50% to 80% inhibition of calcium release and 33% to 60% inhibition of phosphate release in the acid challenge Fluoride paste provided better protection	0.012 0.013	HAP, even at 1%, provided significant protection against enamel erosion	A good in situ test to show erosion protection by HAP  HAP < fluoride
Harks et al. 2016 <sup>51</sup> (Germany)	70 adult subjects (mean age 52.8 years for test, 53.5 years for control)	Biorepair® (HAP toothpaste)	Meridol® (Amine fluoride/stannous fluoride)	RCT on biofilm reduction Randomized, prospective 12 weeks	Daily toothbrushing with control and test paste Biofilm formation rate and other secondary clinical and microbiological parameters recorded	Double blinded	Yes Kappa scores NR	Slight reduction in cultivated bacteria (HAP > fluoride)  O'Leary Plaque Index did not differ significantly	0.051 0.069	Periodontal health improved Plaque scores did not differ between groups	A well-conducted clinical trial HAP paste showed similar plaque scores to the fluoride paste HAP = fluoride
Hegazy & Salama 2016 <sup>52</sup> (Egypt)	81 children (ages 7 years to 12 years)	Biorepair® mouthwash	1. Listerine® fluoride mouthwash 2. Peridex®	RCT on biofilm reduction Randomized 6-week trial with weekly assessments	5 mL mouthwash rinsing 30 sec. 2x/day Gingival and plaque scores obtained DIAGNOdont readings to measure remineralization	Double blinded	Kappa = 0.87, 0.89	41% reduction in plaque index similar to Listerine, Peridex Better remineralization than Listerine and Peridex	0.05 <0.0001 0.02 0.619	Biorepair® and fluoride mouthwashes were equally effective in reducing biofilm and gingivitis as compared to chlorhexidine mouthwash	A well-conducted trial HAP mouthwash reduces biofilm as well as Listerine® with fluoride and Peridex® Subjects were children HAP = fluoride

Table S4. *continued*

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Makeeva et al. 2016 <sup>53</sup> (Russia)	Group 1: 15 subjects (ages 17 years to 25 years) Group 2: 15 subjects (ages 35 years to 44 years)	Apadent®	NR	In vivo trial testing HAP protection against acid erosion Clinical examination after 3 mo. application of test paste	Daily toothbrushing with the test paste Schiff index KOSRE and TER tests on enamel biopsies	NR	NR	Acid resistance significantly increased by 50%	NR	Apadent® increased acid resistance of teeth and reduced sensitivity	Interesting in vivo model using an acid challenge intraorally Showed acid resistance of enamel after HAP application No controls reported
Souza et al. 2015 <sup>54</sup> (Brazil)	240 dentin slabs in 13 subjects (ages 19 years to 28 years)	Nanop Plus (10% HAP, 0.2% NaF)	MI Paste Plus (CPP-ACP with 0.2% NaF) 0.2% NaF Placebo	In situ crossover trial on demin/remin Randomized 14 days each arm of the crossover	240 bovine enamel and dentin blocks mounted on palatal appliances 4 min. topical application of slurries outside the mouth Regular eating and oral hygiene with same toothpaste TMR analysis	Double blinded	NR	2.7-fold increase in tooth remineralization compared to the placebo	0.05	Test treatments did not differ much but Nanop Plus was the only one able to significantly reduce dentin demineralization and to improve enamel remineralization compared to placebo	A well-conducted in situ trial HAP worked better than fluoride to protect dentin surfaces HAP > fluoride
Lelli et al. 2014 <sup>55</sup> (Italy)	10 adult subjects (ages 18 years to 75 years)	Biorepair® (20% zinc-HAP)	Pronamel® (1450 ppm fluoride) toothpaste	In vivo trial on HAP deposition Randomized 5 test and 5 control subjects donated their extracted teeth after the 8 weeks of exposure	Post-extraction examination of the effects of toothpaste SEM, X-ray diffraction and infrared microscopic spectral analysis of enamel surfaces	NR	NR	NR	NR	HAP-rich deposits were found on the enamel surfaces of the test group, not the fluoride group	Low subject numbers Showed HAP adhesion to teeth
Hannig et al. 2012 <sup>56</sup> (Germany)	36 enamel slabs in 6 adult subjects (age NR)	Biorepair® mouthwash (20% zinc-HAP)	Placebo Meridol® (0.2% chlorhexidine)	In situ trial on biofilm effect 6 bovine enamel slabs mounted on each maxillary splint In situ pellicle formation, in vitro artificial caries 1 min. mouthwash exposure, 30 min. intraoral	BacLight bacterial viability staining and fluorescent microscopy after DAPI staining of plaque growth in situ	NR	NR	A significant reduction in bacterial colonization and <i>S. mutans</i> viability with BioRepair compared to the placebo	<0.001 <0.001	The HAP mouthwash inhibited biofilms by interfering with adherence and growth of <i>S. mutans</i>	Asmall study but enough enamel samples to observe significant reductions in biofilm growth and viability
Najibfard et al. 2011 <sup>57</sup> (USA)	120 human enamel blocks worn by 30 adults (mean age 37.8 years)	5% HAP 10% HAP (on demineralized slabs)	Fluoride (1100 ppm) on demineralized slabs) 10% HAP on health enamel slabs	Randomized in situ trial on remineralization 4 slabs attached to one mandibular splint/ subject Brushing 3x/day 4-phase trial, 28 days per phase	Mineral loss and lesion depth measured with transvers microradiography	Double blinded	NR	5% HAP = 66.5% remineralization 10% HAP = 72% remineralization F (1100 ppm) = 68% remineralization	<0.001 <0.001 <0.001	"Dentifrices containing nHAP have the potential for the same remineralizing capacity as a fluoride dentifrice"	A well-designed in situ study  HAP = fluoride

NR: not reported; NA: not applicable

REFERENCE Limeback H, Enax, J, Meyer F. Biomimetic hydroxyapatite and caries prevention: a systematic review and meta-analysis. *Can J Dent Hyg.* 2021;55(3):148-59.