

1 **Impact of acquired pellicle modification on adhesion of early colonizers**

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21 Disclosure Statement

22 The authors have no conflicts of interest to declare.

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25 Abstract

26 New preventive approaches against dental erosion caused by acidic drinks and beverages
27 include fortification of beverages with natural polymers. We have shown that the mixture of
28 casein and mucin significantly improved the erosion-inhibiting properties of the human
29 pellicle layer. This study aimed to investigate the effect of pellicle modification by casein,
30 mucin and a casein/mucin mixture on the adhesion of early bacterial colonizers. Test
31 specimens of human tooth enamel were prepared, covered with saliva and coated with 0.5%
32 aqueous casein, 0.27% aqueous mucin or with 0.5% aqueous casein/0.27% aq. mucin, after
33 which the adhesion of *Streptococcus gordonii*, *Streptococcus oralis*, and *Actinomyces*
34 *odontolyticus* was measured after incubation for 30 min and 2 h. Log₁₀ colony forming units
35 were compared by non-parametric tests. All three bacterial strains adhered in higher number
36 to pellicle-coated enamel than to native enamel. The protein modifications of pellicle all
37 decreased the counts of adhering bacteria up to 0.34 log₁₀ /mm², the most efficient being
38 casein/mucin mixture. In addition to the recently shown erosion-reducing effect by
39 casein/mucin, modification of the pellicle may inhibit bacterial adherence compared to
40 untreated human pellicle.

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43 Caries is associated with bacterial processes leading to damage of the hard dental tissues
44 (enamel, dentine and cementum) and remains one of the most common diseases worldwide
45 [Kassebaum et al., 2015] Caries results from the interplay of three main factors: dietary
46 carbohydrates, cariogenic bacteria on the dental surface and susceptibility of hard tooth
47 tissues to demineralization. [Bradshaw and Lynch, 2013; Kutsch and Young, 2011].

48 The prevalence of dental erosion is steadily increasing in European countries and requires
49 effective preventive solutions [Jaeggi and Lussi, 2014]. It is widely documented that acidic
50 beverages as well as gastric juice may cause dental erosion [Lussi et al., 1995; Lussi et al.,
51 2012; Sovik et al., 2015; Wilder-Smith et al., 2015]. New approaches to preventing dental
52 erosion, which include fortification of beverages with natural polymers, or with dietary
53 proteins such as ovalbumin from egg white, have been reported to reduce acid-induced
54 hydroxyapatite dissolution [Barbour et al., 2008; White et al., 2011]. Furthermore, when
55 added to commercial soft drinks or simple acidic solutions, casein subfractions (α -, β - and κ -)
56 and ovalbumin reduced enamel erosion in the presence of the acquired pellicle [Hemingway
57 et al., 2008]. The addition of dietary proteins to soft drinks is currently regarded as a
58 promising preventive measure as it does not spoil the taste and carries no risk of dental
59 staining. We proved in our previous study that modification of salivary pellicle by casein and
60 casein/mucin mixture (mucin–salivary protein) increased the ability of the pellicle layer to
61 protect against erosive enamel demineralization [Cheaib and Lussi, 2011]. Interestingly,
62 application of casein–mucin mixture for pellicle modification showed greater anti-erosive
63 efficacy than application of casein alone. In spite of the proven efficiency of these proteins in
64 the inhibition of enamel demineralization and wear, it is not clear if incorporating them into
65 the pellicle layer can also interfere with bacterial adhesion provoking or preventing caries.
66 Thus, it is necessary to assess the preventive effect of proteins against dental erosion as well
67 as against caries. Such complementary studies are much in demand in this field.

68 Bacterial colonization starts with the adhesion of early colonizers, known as pioneer bacteria,
69 to the salivary pellicle of the teeth, as well as of the dental restorations, within minutes after
70 tooth cleaning. These early colonizers, mostly streptococci and *Actinomyces* spp., contribute
71 to plaque development and ultimately to oral diseases [Li et al., 2004]. The bacterial adhesion
72 occurs via specific receptors located in the salivary pellicle layer and follows the typical
73 stages of biofilm formation [Marsh, 2004].

74 The molecular composition and physicochemical properties of the pellicle are critical in
75 determining the pattern of microbial colonization. As bacteria approach the pellicle layer
76 weak physicochemical forces are generated. Within a short time these weak physicochemical
77 interactions may become irreversible due to adhesins on the microbial cell surface, which are
78 involved in specific interactions with complementary receptors [van der Mei et al., 2008].
79 Thus, a key event in biofilm formation is the initial adhesion of bacteria to matching host
80 ligands.

81 The aim of this study was to investigate the effect of pellicle modification by casein, mucin
82 and a casein/mucin mixture being a potential alternative in preventing dental erosion on the
83 adhesion of early bacterial colonizers *Streptococcus gordonii*, *Streptococcus oralis* and
84 *Actinomyces odontolyticus*.

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86

87 Materials and Methods

88 The bacteria used for this study were: *Streptococcus gordonii* ATCC 10558, *Streptococcus*
89 *oralis* ATCC 35037 and *Actinomyces odontolyticus* ATCC 17982. The strains were
90 maintained on tryptic-soy-agar (Oxoid, Basingstoke, GB) with 5% sheep blood. They were
91 always passaged 16 h before the experiment.

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94 Saliva collection

95 Whole saliva was collected from healthy volunteers by paraffin stimulation and pooled. Saliva
96 collection was approved by the ethics committee of the University of Bern. To remove debris
97 the saliva was centrifuged for 20 minutes at 4 °C and 4,000 g. The supernatant was sterilized
98 using UV radiation for 30 minutes. The processed, sterile saliva sample (tested by cultivation)
99 was then stored at -80 °C; aliquots of saliva were defrosted immediately before the
100 experiment.

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103 Preparation of human tooth specimens

104 After patients had given informed consent for the use of their extracted teeth for research
105 purposes (in accordance with the regulations of the ethics committee of the University of
106 Bern), cavity-free human premolars were selected and viewed under a stereomicroscope
107 (Leica, Zoom 2000, USA; 25× magnification) to choose specimens with a sound undamaged
108 surface. Tooth crowns were separated from the roots using an IsoMet® Low Speed Saw
109 (Buehler, Düsseldorf, Germany). The buccal sites were then divided into small cubical slabs,
110 and polished on a Kuntch-Rotor polishing machine with carbide paper (30 µm, 18 µm and
111 6 µm grain size) under water cooling. The cubes had a size of about $2 \times 2 \times 0.75$ mm and
112 consist of enamel only (checked by stereomicroscopy).

113 Samples were stored in a mineral solution (1.5 mmol/l CaCl_2 , 1.0 mmol/l KH_2PO_4 , 50 mmol/l
114 NaCl, pH 7.0). Prior to the experimental procedures, the samples were disinfected in ethanol
115 (70%) for 1 h, and washed in sterile deionized water for a further 1 h.

116

117 Calculation of specimen surface area

118 The determination of surface area was performed using a light microscope (Leica M 420).
119 Every side of each tooth specimen was manually outlined using Software IM500, and the
120 surface was measured taking into account the corresponding magnification factor of the
121 microscope. The total surface area was calculated as the sum of the areas of all the faces.

122

123 In vitro bacterial adhesion assay

124 For each bacterial strain, the enamel specimens were randomly selected and divided into eight
125 groups: 1) native enamel (E, n=20); 2) pellicle layer (P, n=20); 3) enamel incubated in 0.5%
126 aq. casein ((casein from bovine milk, Merck for analysis) C, n=20); 4) enamel incubated in
127 0.27% aq. mucin ((gastric mucin from pig, Merck for analysis) M, n=20); 5) enamel
128 incubated in 0.5% aq. casein/0.27% aq. mucin (CM, n=20); 6) pellicle incubated in casein
129 0.5% aq. (PC, n=20); 7) pellicle incubated in 0.27% aq. mucin (PM, n=20); 8) pellicle
130 incubated in 0.5% aq. casein/0.27% aq. mucin (PCM, n=20). The chosen concentrations are
131 based on the results of the study made before and provide buffering potential similar to human

132 salivary proteins [Cheaib and Lussi, 2011]. To prepare protein and protein-modified pellicle
133 coatings, enamel was incubated in the corresponding aqueous solution of the proteins casein,
134 mucin and casein/mucin mixture for 2 h, or in sterile human saliva for 2 h followed by protein
135 aqueous solution for 2 h for pellicle coated by protein groups (or left in saliva for 2 h), at
136 room temperature under sterile conditions immediately before starting the experiments.
137 Samples were then contaminated with bacterial suspension (OD₆₀₀=1.05; equivalent to 10⁸
138 bacteria/ml) for 30 min or 2 h in Dulbecco's modified Eagle's medium (DMEM; Gibco,
139 Invitrogen) under slow motion irrigation before being carefully dipped in DMEM to eliminate
140 non-adhering bacteria. Samples were soaked in NaCl 0.9%, and bound bacteria were
141 dispersed by sonication for 30 s. The samples were vortexed for a further 30 s, serially diluted
142 and spread over a tryptic-soy-agar plate. The media were incubated at 37 °C with 5% carbon
143 dioxide for 48 h and bacterial colonies were then counted using an Acolyte Super Count
144 Colony Counter (Synbiosis, UK). The number of bacteria was measured as colony-forming
145 units (CFU). Results are expressed as log₁₀ CFU per mm². Each two specimens per coating
146 and time were independently included in one run. Meaning, at least 10 runs per bacterial
147 strain were conducted.

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150 Statistical analysis

151 All statistical analysis was performed with R, Version 2.15.1, using the extension package
152 exact RankTests. A global significance level of alpha = 0.05 had to be achieved.

153 Since the data did not fulfil the assumptions of a parametric ANOVA (heteroskedastic groups,
154 i.e. unequal variances), a nonparametric approach known as the aligned rank transformation
155 (ART) method (Higgins 2004) was applied [Higgins, 2004]. Comparisons between different
156 groups were made by using Kruskal-Wallis test.

157

158 Results

159 Eight groups at two different sampling times per bacterial strain (n=20/group) were compared
160 in the experiment. Slabs of native enamel, enamel slabs coated by a pellicle layer, by casein,
161 mucin and casein/mucin mixture, and enamel slabs coated first by pellicle layer followed by

162 casein, mucin and casein/mucin mixture were prepared. All test groups were incubated for 30
163 min or 2 h with *S. gordonii* ATCC 10558, *S. oralis* ATCC 35037 or *A. odontolyticus* ATCC
164 17982.

165

166 Adhesion of *Streptococcus gordonii* ATCC 10558 to native enamel and pellicle- 167 covered enamel with different coatings

168 The adhesion rate of *S. gordonii* ATCC 10558 was significantly higher to pellicle coated
169 enamel than to native enamel after 30 min and 2 h incubation ($p=0.017$; $p<0.001$). After 30
170 min, coating with casein and with casein/mucin decreased the adhesion of *S. gordonii*
171 ($p=0.006$; $p=0.005$) compared to native enamel and after 2 h incubation there was still less
172 adhesion after casein/mucin coating ($p=0.040$) (all: $p=0.010$, $p=0.155$). When comparing
173 pellicle-covered enamel, all modifications significantly decreased the adhesion of *S. gordonii*
174 after 30 min (casein $p=0.002$, mucin and casein/mucin both $p<0.001$) and after 2 h the lowest
175 rate was found after casein/mucin with a reduction in median by up to 0.43 log₁₀ CFU (or
176 63%, $p=0.001$) (all: $p<0.001$, $p=0.003$). However, counts were higher when comparing
177 modified pellicle with native enamel coated with the respective proteins (casein after 30 min
178 and 2 h ($p<0.001$; $p=0.006$), mucin and casein/mucin after 2 h ($p=0.011$; $p=0.009$) (figure 1).

179

180 Adhesion of *Streptococcus oralis* ATCC 35037 to native enamel and pellicle- 181 covered enamel with different coatings

182 The adhesion rate of *S. oralis* ATCC 35037 was significantly higher in pellicle-coated enamel
183 than in native enamel after 30 min and 2 h incubation ($p<0.001$; $p=0.001$). After 30 min and
184 2 h incubation, coating with casein ($p=0.002$; $p=0.013$), mucin ($p=0.034$; $p=0.002$) and with
185 casein/mucin ($p<0.001$; $p=0.006$) decreased the adhesion of *S. oralis* compared to native
186 enamel (all: $p=0.001$, $p=0.009$). With regard to pellicle-covered enamel, mucin and
187 casein/mucin modifications significantly decreased the adhesion of *S. oralis* after 30 min
188 ($p=0.038$; $p<0.001$), the difference was still significant for casein/mucin after 2 h ($p=0.020$)
189 (all: $p=0.001$, $p=0.057$). All bacterial counts were higher when comparing modified pellicle

190 with native enamel coated with the respective proteins (each $p < 0.001$) after 30 min and 2 h of
191 incubation (figure 2).

192

193 Adhesion of *Actinomyces odontolyticus* ATCC 17982 to native enamel and 194 pellicle-covered enamel with different coatings

195 The adhesion rate of *A. odontolyticus* ATCC 17982 was significantly higher in pellicle-coated
196 enamel than in native enamel after 30 min and 2 h incubation (both $p < 0.001$). Coating with
197 casein, mucin or with casein/mucin mixture did not change the adhesion of *A. odontolyticus* to
198 native enamel (all: $p = 0.348$, $p = 0.247$). In the case of pellicle-covered enamel, modifications
199 with casein significantly decreased the counts of *A. odontolyticus* after 30 min ($p = 0.009$) and
200 modifications with mucin after 2 h ($p = 0.004$). Casein/mucin mixture inhibited bacterial
201 adhesion after 30 min ($p = 0.003$) and 2 h ($p < 0.001$) (all: $p = 0.011$, $p = 0.001$). Counts were
202 higher when comparing modified pellicle with native enamel coated with the proteins (casein
203 after 2 h ($p = 0.020$), mucin after 30 min and 2 h ($p < 0.001$; $p = 0.006$) and casein/mucin after 30
204 min ($p = 0.015$) (figure 3).

205

206 Comparison between adhesion of bacterial strains after 30 min and 2 h 207 incubation

208 After 2 h, the counts of attached bacteria were always higher than after 30 min; the difference
209 ranged between $0.14 \log_{10} \text{ cfu/mm}^2$ (*S. oralis* ATCC 35037 coating with casein) and 0.59
210 $\log_{10} \text{ cfu/mm}^2$ (*S. oralis* ATCC 35037 coating with casein/mucin).

211 The rate of bacterial adhesion to native and pellicle-coated enamel with and without
212 modifications was always the highest for *A. odontolyticus* 17982. The rate was always
213 significantly different ($p < 0.001$) from those of *S. oralis* ATCC 35037 and *S. gordonii* ATCC
214 10558, both after 30 min and after 2 h. Furthermore, *S. oralis* adhered in greater numbers than
215 *S. gordonii* to native enamel at 2 h ($p = 0.007$), and to pellicle-covered enamel with and
216 without modifications after 30 min (without modifications $p = 0.001$; casein and mucin both
217 $p < 0.001$, casein/mucin 0.003) and after 2 h (without modifications $p = 0.026$; casein $p < 0.001$;
218 mucin $p = 0.001$, casein/mucin 0.006). For native enamel coated with casein/mucin, after 30

219 min, *S. gordonii* adhered in greater numbers than *S. oralis* (p=0.007). There was no difference
220 between the adherence of the two streptococcal strains on native enamel after 30 min, native
221 enamel coated with casein and mucin at either time and native enamel coated with
222 casein/mucin mixture after 2 h.

223

224 Discussion

225 We studied the effect of pellicle modification by casein, mucin or a mixture of the two on
226 formation of biofilm on human enamel. Our previous study showed that the mixture of casein
227 and mucin increased the original ability of pellicle to protect against dental erosion [Cheaib
228 and Lussi, 2011].

229 Microbial adhesion on tooth surfaces is always preceded by the adsorption of a salivary
230 pellicle [Hannig and Joiner, 2006; Lendenmann et al., 2000]. After adhering, the early
231 colonizers such as *Actinomyces* spp., *S. oralis*, *Streptococcus mitis*, or *S. gordonii* influence
232 the formation and the composition of the mature [Kolenbrander, 2000]. Thus, they provide
233 optimal conditions and attachment substrates for the secondary colonizers, i.e., *Streptococcus*
234 *mutans*, *Fusobacterium nucleatum*, or *Veillonella* spp. In this study, *S. gordonii* ATCC 10558,
235 *S. oralis* ATCC 35037 and *A. odontolyticus* ATCC 17982 were used for evaluating early
236 biofilm formation on the protein-modified enamel and protein-modified salivary pellicle. The
237 number of adhered bacteria was higher in the pellicle-coated enamel group than in the native
238 enamel group after 30 min and 120 min incubation. This could be explained by nonspecific
239 and specific interactions which operate together on the pellicle layer leading to a stronger
240 adhesion of *S. gordonii* ATCC 10558, *S. oralis* ATCC 35037 and *A. odontolyticus* ATCC
241 17982 to the pellicle surface. This is in agreement with our previous study [Mai et al., 2014]
242 showing that pellicle promotes more bacterial adhesion than human enamel.

243 The primary interactions of bacteria with the tooth surface can result from diffusion,
244 sedimentation, liquid flow, or from active movement of bacteria. Interactions between the
245 surface and the bacteria include electrostatic, hydrophobic, and van der Waals forces, and lead
246 to firm nonspecific but reversible adhesion of the bacteria to the pellicle [Kolenbrander, 2000]
247 The irreversible attachment of bacterial cells to the tooth involves specific, stereochemical
248 interactions between bacterial adhesins and complementary receptors in the acquired pellicle.

249 Furthermore, specific interactions strengthen more over more extended periods of time than
250 nonspecific ones [van der Mei et al., 2008]

251 The number of bacteria that had adhered to pellicle and protein-coated pellicle was higher for
252 *A. odontolyticus* ATCC 17982 than *S. gordonii* ATCC 10558 and *S. oralis* ATCC 35037 after
253 both 30 min and 2 h incubation. This could be explained by stronger nonspecific and specific
254 interactions with *A. odontolyticus* ATCC 17982 than for the two other strains. *Actinomyces*
255 spp. have two distinct types of fimbriae: type 1 fimbriae mediate adherence to proline-rich
256 proteins and to statherin, whereas type 2 fimbriae are involved in the adherence of bacterial
257 cells to already attached bacteria [Hallberg et al., 1998]. *A. odontolyticus* expresses a sialic
258 acid binding specificity potentially related to type-2 fimbriae [Drobni et al., 2006]. This
259 specific interaction with the protein layer via fimbriae may result in stronger binding sites.
260 Adhesins on *S. gordonii* can bind to α -amylase [Rogers et al., 2001]. *S. gordonii* and *S. oralis*
261 express LPXTG-linked adhesins which binds to saliva-coated surfaces [Davies et al., 2009;
262 Dorkhan et al., 2012]. The 30 min and 2 h incubation times in this study were chosen to
263 acquire first-hand knowledge about nonspecific and specific interactions of bacteria with
264 protein-modified enamel and pellicle.

265 For *S. gordonii* ATCC 10558, smaller numbers of bacteria adhered to pellicle modified by
266 casein, mucin or a mixture of the two, or to enamel modified by proteins, than to non-
267 modified pellicle. The difference was statistically significant at the two incubation times.
268 Also, for *A. odontolyticus* ATCC 17982, the number of adhered bacteria was lower in casein-
269 coated pellicle after 30 min incubation and in mucin-coated pellicle after 2 h incubation
270 compared to pellicle layer. For *S. oralis* ATCC 35037, incubation of enamel in saliva
271 followed by protein treatment resulted in lower counts in the casein/mucin-coated pellicle
272 group than in the pellicle group.

273 Nonspecific interactions are mainly altered upon addition of casein to pellicle layer, as the
274 effect had already occurred at 30 min. However, in the case of *A. odontolyticus* ATCC 17982,
275 specific interactions may be affected upon addition of mucin. The modification of pellicle by
276 casein/mucin mixture resulted in lower numbers of all the bacteria tested than counted for
277 pellicle at the two incubation times. Casein forms micelle-like structures on the pellicle
278 [Cheaib and Lussi, 2011]. The addition of casein promoted adsorption of mucin and it is
279 known that mucin interacts weakly with the pellicle layer on salivary pellicle in vitro [Cheaib

280 and Lussi, 2011]. Therefore, the formation of a protein complex between casein and mucin
281 and its interaction with pellicle could explain a better shielding barrier between the pellicle
282 layer and oral bacterial cells.

283 We studied adhesion of initial colonizers to modified pellicle. Independently of the used strain,
284 application of pellicle increased bacterial counts by 0.13 - 0.34 log₁₀ cfu/mm² and
285 modification by 0.27% mucin/0.5% casein reduced bacterial adhesion up to 0.33 log₁₀
286 cfu/mm². This suggests a non-selective effect of 0.27% mucin/0.5% casein resulting in a
287 slight inhibition of bacterial adhesion. Following a disturbance of bacterial homeostasis
288 might be excluded. Bacterial homeostasis in the oral cavity include the integrity of host
289 defence and the consumption of sugars [Marsh, 2006]. Pulsing of a microbial community with
290 glucose in uncontrolled pH conditions led to a decrease of acid-sensitive microorganisms
291 among them *S. gordonii* and *S. oralis* by favoring acidogenic bacteria e.g. *S. mutans*
292 [Bradshaw and Marsh, 1998]. Sequence analysis of severe caries confirmed a decrease of *S.*
293 *gordonii*, while *S. mutans* increased and *Actinomyces* sp. remained unchanged [Gross et al.,
294 2010]. Casein inhibits the adherence of cariogenic bacteria to saliva-coated surfaces. Sodium
295 caseinate, casein phosphopeptide, and glycol-macropeptide inhibited adherence of oral
296 streptococci to saliva-coated hydroxyapatite beads. Anticariogenicity of these proteins and
297 peptides was accomplished by selectively inhibiting adhesion of streptococci to the teeth;
298 microbial composition of dental plaque was modulated to favour establishment of less
299 cariogenic species, such as oral *Actinomyces* spp. [Neeser et al., 1994]. Milk and individual
300 caseins (α , β , and κ caseins) were studied to determine adherence of *S. mutans* to saliva-
301 coated hydroxyapatite discs. Milk inhibited in vitro adherence of *S. mutans* GS-5 [Vacca-
302 Smith et al., 1994]. Individual caseins were also examined. No effect on streptococcal
303 adherence was observed when α - or β -casein was incubated with hydroxyapatite beads.
304 However, κ -casein inhibited adherence of *S. mutans* GS-5. Inhibitory properties were
305 attributed to a 40 kDa glycoprotein [Vacca-Smith et al., 1994]. It has also been reported that
306 α -, β - and κ - caseins of bovine origin inhibit adhesion of *S. mutans* to saliva-coated
307 hydroxyapatite [Malkoski et al., 2001]. Another study, however, found clear inhibition of
308 adhesion of *S. mutans* strains to saliva-coated hydroxyapatite by the C-terminal fraction of β -
309 casein [Danielsson Niemi et al., 2009].

310 In a study in rats, micellar casein was shown to prevent oral colonization by *Streptococcus*
311 *sobrinus* and to promote colonization by *Actinomyces viscosus*. Sodium caseinate was not as
312 effective as micellar casein at inhibiting streptococcal colonization [Guggenheim et al., 1999].

313 Our adhesion model used sterilized human saliva to provide a close representation of the
314 natural growth conditions. It has been suggested that bacterial attachment in the mouth is
315 sensitive to the ionic strength of saliva. The ionic strength used in this study was 15 mM for
316 mucin and 11 mM for casein, which is in line with the strengths used in previous studies [Mai
317 et al., 2014]. The present in vitro study has some limitations because it investigated only the
318 simple adhesion of bacterial strains without considering the influence of other strains. To have
319 standardized conditions, pooled saliva from healthy subjects was used. Saliva from healthy
320 subjects and patients with dental erosion do not differ in the total protein amount [Bardow et
321 al., 2014; Carpenter et al., 2014], however the content of calcium and proteins is reduced in
322 newly formed acquired pellicle on enamel in dental erosion [Carpenter et al., 2014].

323 Recently we have shown that pellicle formed with saliva containing a mixture of 0.27%
324 mucin and 0.5% casein clearly inhibits loss of microhardness of enamel already after two
325 cycles of 2 h incubation when being exposed to a 0.65% citric acid solution [Cheaib and
326 Lussi, 2011]. This modification of the pellicle did not only reduce erosion of enamel [Cheaib
327 and Lussi, 2011] but also slightly reduced bacterial adherence compared to untreated human
328 pellicle. This may be of practical significance in prevention, as on the one hand bacterial
329 homeostasis might be not seriously affected and on the other hand as both erosion and caries
330 are widespread around the world. More extensive investigations are required to understand the
331 interaction of a complex oral bacterial community incl. cariogenic bacteria with the 0.27%
332 mucin/0.5% casein mixture as a potential additive to soft drinks in preventing dental erosion.

333

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338

339 **Author Contributions**

340 Z. Cheaib, E. Rhakmatulina, A. Lussi and S. Eick conceived and designed the study. Z.
341 Cheaib performed the experiments. Z. Cheaib, E. Rhakmatulina, A. Lussi and S. Eick
342 analysed the data. Z. Cheaib, E. Rhakmatulina, A. Lussi and S. Eick wrote the manuscript.

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437 Legends

438 **Figure 1**

439 Number (minimum, maximum, quartiles) of *Streptococcus gordonii* ATCC 10558 adhering to
440 different surfaces: native enamel (E), pellicle-covered enamel (P-E), enamel coated with
441 casein (C), enamel coated with mucin (M), enamel coated with casein/mucin (CM), enamel
442 covered with pellicle modified by casein (PC), enamel covered with pellicle modified by
443 mucin (PM) and enamel covered with pellicle modified by casein and mucin (P-CM) after 30
444 min (A) and 2 h (B) incubation. * p<0.05; ** p<0.01; *** p<0.001 pellicle-covered enamel each
445 compared to the same coating without pellicle

446 ¶ p<0.05; ¶¶ p<0.01 coating each compared to enamel

447 † p<0.05; †† p<0.01; ††† p<0.001 coating each compared to pellicle-covered enamel

448

449

450 **Figure 2**

451 Number (minimum, maximum, quartiles) of *Streptococcus oralis* ATCC 35037 adhering to
452 different surfaces: native enamel (E), pellicle-covered enamel (P-E), enamel coated with
453 casein (C), enamel coated with mucin (M), enamel coated with casein/mucin (CM), enamel
454 covered with pellicle modified by casein (PC), enamel covered with pellicle modified by
455 mucin (PM) and enamel covered with pellicle modified by casein and mucin (P-CM) after 30
456 min (A) and 2 h (B) incubation.

457 * p<0.05; ** p<0.01; *** p<0.001 pellicle-covered enamel each compared to the same coating
458 without pellicle

459 ¶ p<0.05; ¶¶ p<0.01; ¶¶¶ p<0.001 coating each compared to enamel

460 † p<0.05; ††† p<0.001 coating each compared to pellicle-covered enamel

461

462 **Figure 3**

463 Number (minimum, maximum, quartiles) of adhered *Actinomyces odontolyticus* ATCC 17982
464 to different surfaces surfaces: native enamel (E), pellicle-covered enamel (P-E), enamel
465 coated with casein (C), enamel coated with mucin (M), enamel coated with casein/mucin
466 (CM), enamel covered with pellicle modified by casein (PC), enamel covered with pellicle
467 modified by mucin (PM) and enamel covered with pellicle modified by casein and mucin (P-
468 CM) after 30 min (A) and 2 h (B) incubation.

469 * p<0.05; ** p<0.01; *** p<0.001 pellicle-covered enamel each compared to the same coating
470 without pellicle

471 †† p<0.01; †††p<0.001 coating each compared to pellicle-covered enamel

472

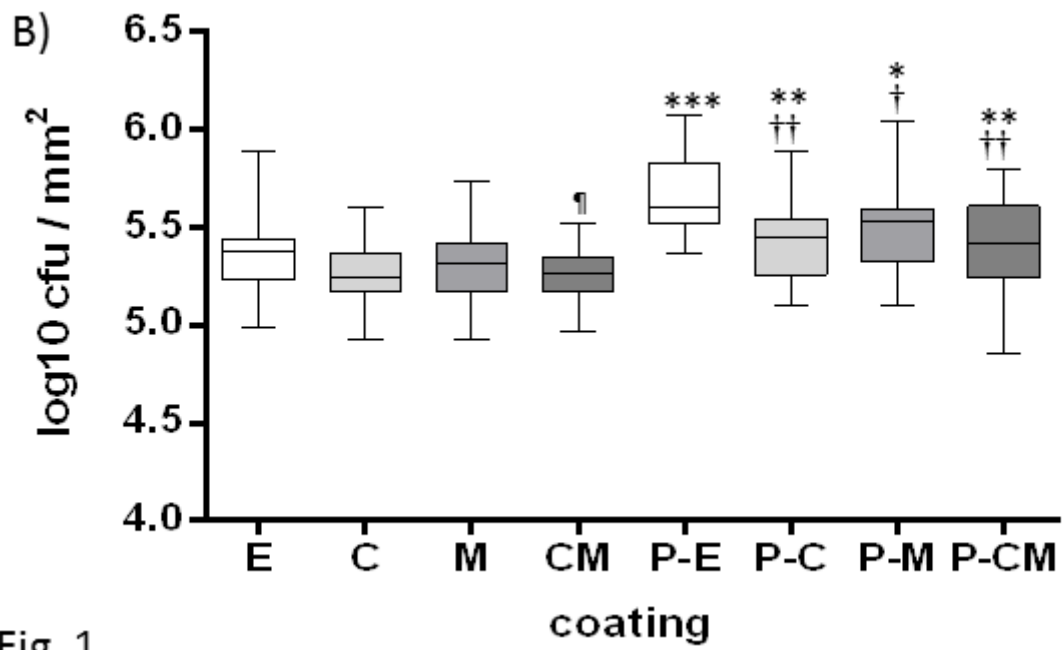
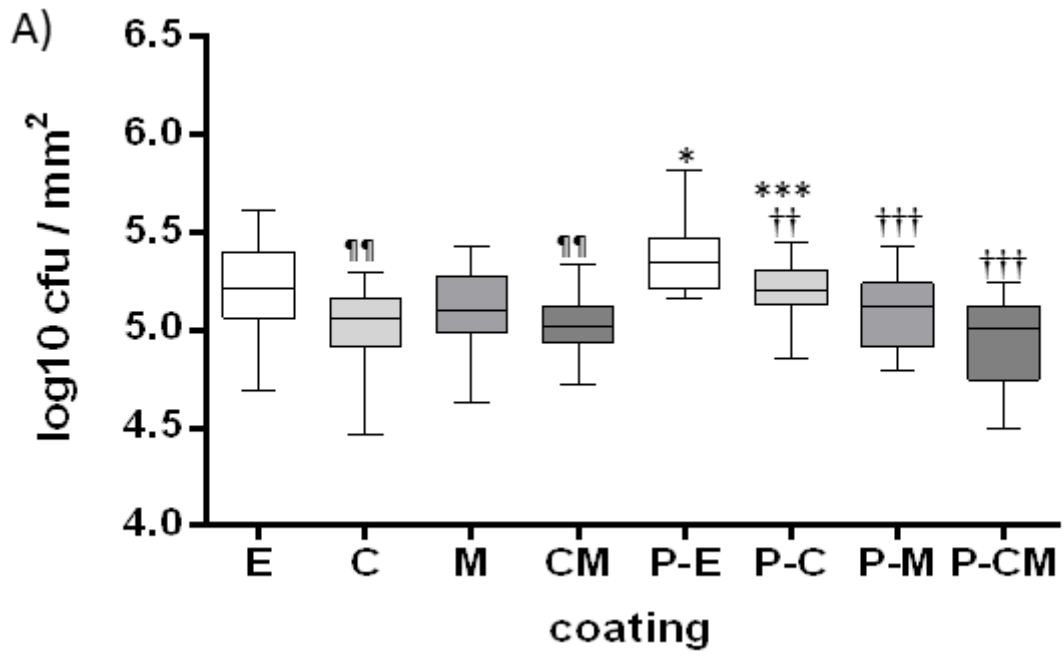


Fig. 1

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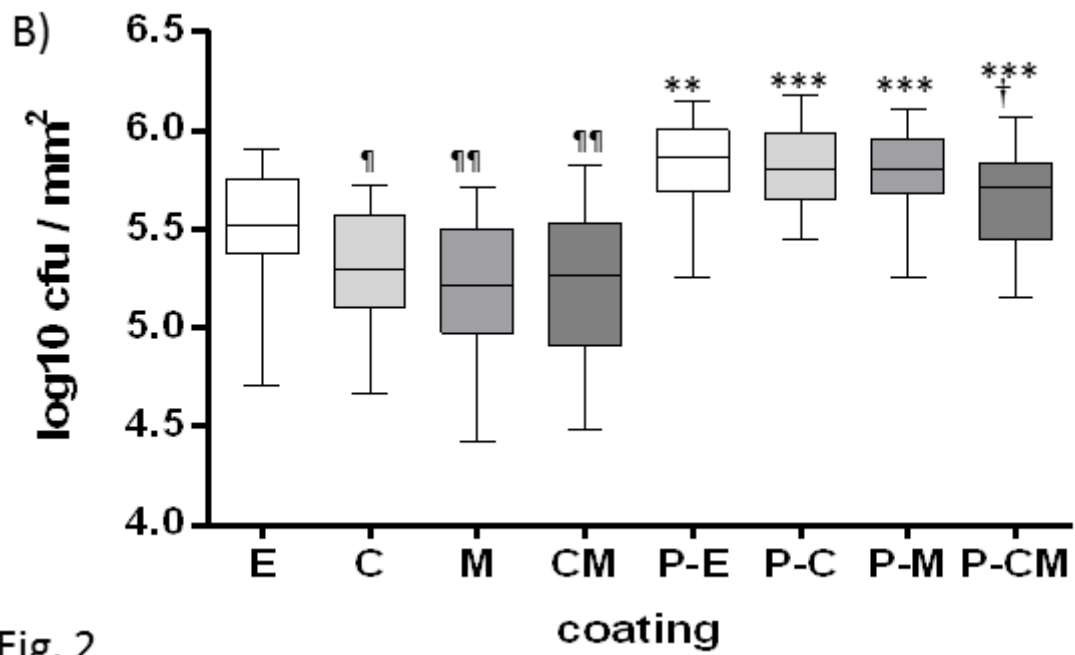
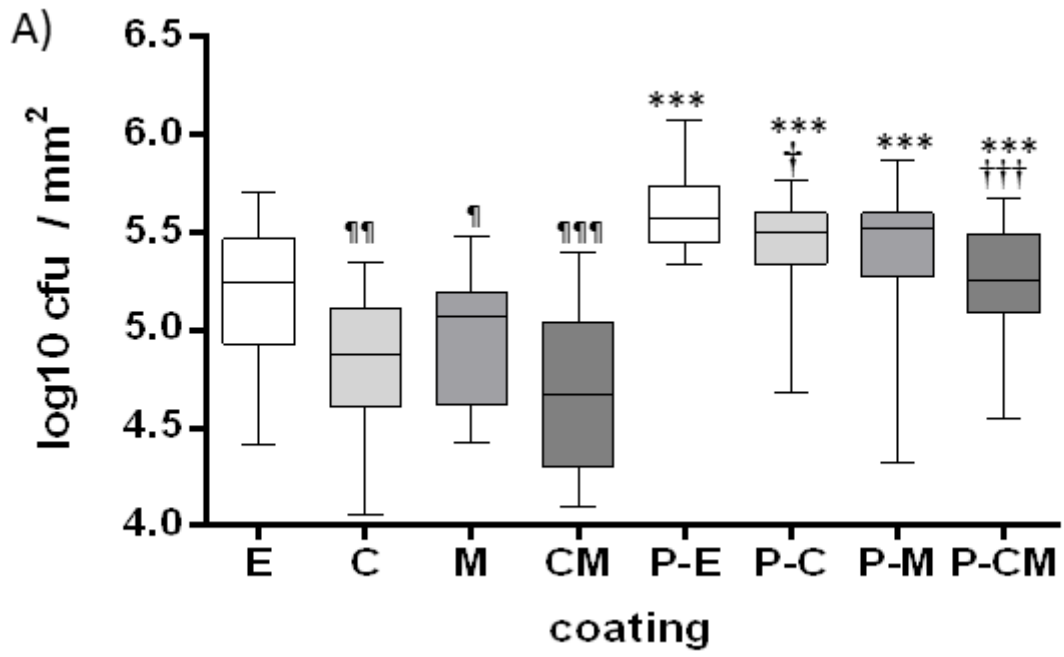


Fig. 2

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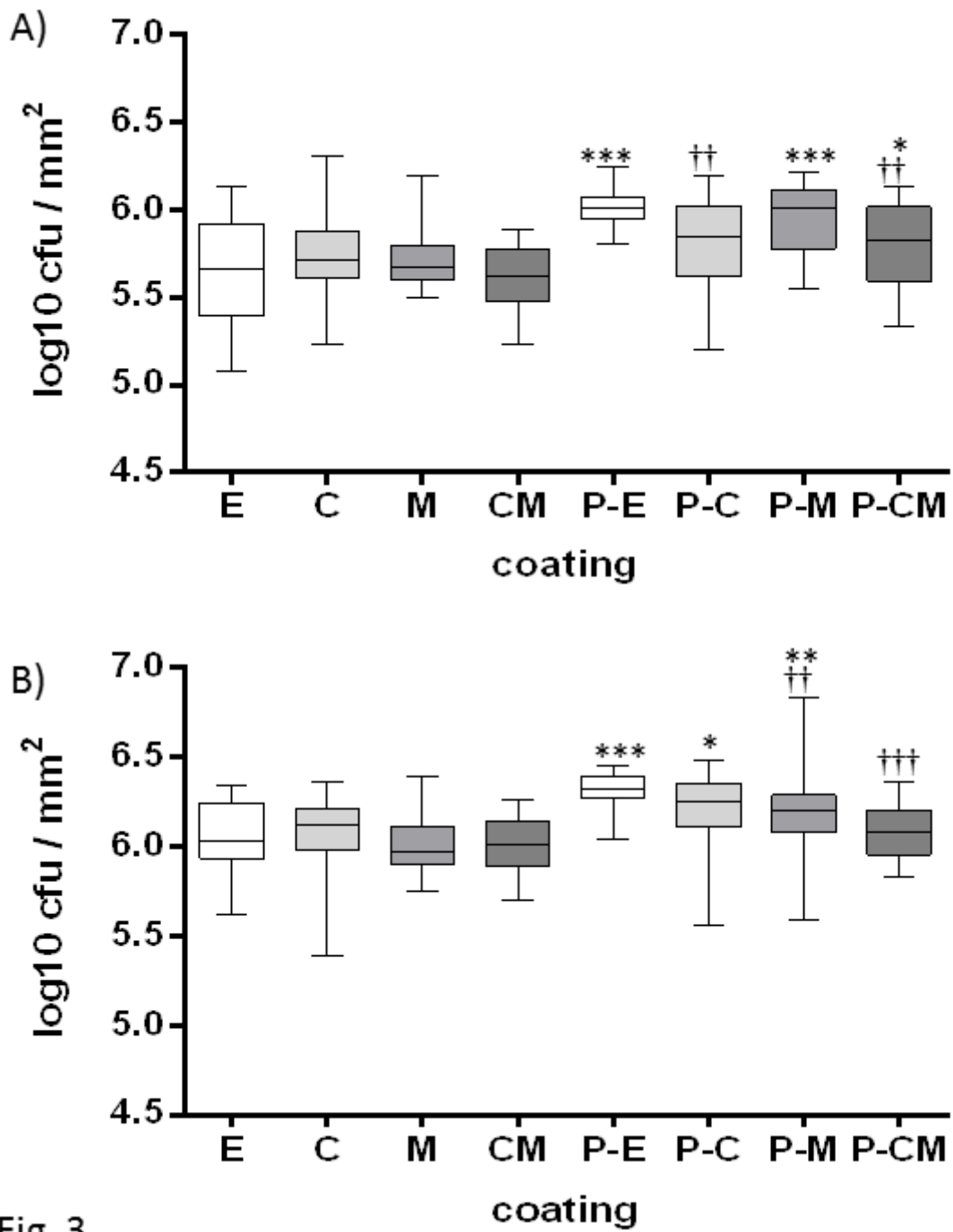


Fig. 3

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