The influences of N-acetyl cysteine (NAC) on the cytotoxicity and mechanical properties of Poly-methylmethacrylate (PMMA)-based dental resin

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Objectives: This study aimed to investigate the influences of N-acetyl cysteine (NAC) on cytotoxicity and mechanical properties of Poly-methylmethacrylate (PMMA) dental resins. **Methods:** Experimental PMMA resin was prepared by incorporating various concentrations of NAC (0, 0.15, 0.3, 0.6 and 0.9 wt.%). MTT assay was performed to investigate viability of human dental pulp cells after exposure to extract of PMMA resin with or without NAC. Cell adhesion on resin specimens was examined with scanning electron microscopy. Degree of conversion was studied with Fourier Transform Infrared Spectroscopy (FTIR). Flexural strength, microhardness and surface roughness was evaluated using a universal testing machine, microhardness tester and optical profilometer, respectively. **Results:** Incorporation of NAC into PMMA resin significantly reduced its cytotoxicity and enhanced cell adhesion on its surface. NAC induced negative influences on the mechanical and physical properties of PMMA resin in a dose-dependent manner. The degree of conversion for all experimental PMMA resins reached as high as 72% after 24 h of polymerization. All the tested properties were maintained when the concentration of incorporated NAC was 0.15 wt.%. **Conclusion:** The addition of 0.15 wt.% NAC remarkably improved biocompatibility of PMMA resin without exerting significant negative influence on its mechanical and physical properties.

The Influences of N-acetyl cysteine (NAC) on the Cytotoxicity and Mechanical Properties of Poly-methylmethacrylate (PMMA)-based Dental Resin

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32 Transform Infrared Spectroscopy (FTIR). Flexural strength, microhardness and surface roughness was

33 evaluated using a universal testing machine, microhardness tester and optical profilometer, respectively.

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36 cell adhesion on its surface. NAC induced negative influences on the mechanical and physical

37 properties of PMMA resin in a dose-dependent manner. The degree of conversion for all experimental

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39 maintained when the concentration of incorporated NAC was 0.15 wt.%.

40 **Conclusion:** The addition of 0.15 wt.% NAC remarkably improved biocompatibility of PMMA resin

41 without exerting significant negative influence on its mechanical and physical properties.

42 **Key words:** poly-methylmethacrylate (PMMA), N-acetyl cysteine (NAC), detoxification, cell viability,

43 mechanical properties

Introduction

Self-polymerizing poly-methylmethacrylate (PMMA)-based dental resin, consisting of a polymer powder and a liquid monomer, is one of the most frequently-used materials in dentistry (Burns et al. 2003). It can be used for fabrication of temporary crowns, denture repair, and the temporary seal of prepared cavities. There have been concerns that when PMMA resin is loaded directly onto prepared teeth, such as in the case of temporary crown fabrication, unpolymerized monomers released from the resin may invade into the dental pulp through dentinal tubules and thus induce negative influences on the pulp tissue (Inoue et al. 2001; Noda et al. 2002).

The cytotoxic effects of various dental monomers have been well-documented. Although the detailed mechanism is still largely unknown, there is striking evidence indicating that the disturbance of intracellular redox balance is related to monomer-related cytotoxicity (Ciapetti et al. 2002; Huang & Chang 2005; Ratanasathien et al. 1995; Vale et al. 1997). It has been reported that dental monomers reduced the intracellular levels of glutathione (GSH), an important non-enzymatic anti-oxidant that protects the cell against oxidative stress, and thus induces the over-production of reactive oxygen species (ROS). Such disturbance of cellular redox balance would eventually result in cell function abnormality and even cell death (Bakopoulou et al. 2009; Schweikl et al. 2006).

Recently, N-Acetyl Cysteine (NAC), a well-known antioxidant, has been found effective in protecting cells against dental monomer-related cytotoxicity (Lee et al. 2006; Paranjpe et al. 2008a; Paranjpe et al. 2008b; Schweikl et al. 2007; Spagnuolo et al. 2006). When incorporated in PMMA resins, NAC restored cell viability and function to a biologically significant degree (Att et al. 2009; Kojima et al. 2008; Yamada et al. 2009; Yamada et al. 2008). Considering that mechanical properties are also important factors that determine the clinical success of PMMA resins, it is necessary to study the influences of NAC on various mechanical and physical properties of PMMA resins. However, up to now, there is limited published data regarding this aspect. Although Wael et al. demonstrated that the addition of NAC up to 0.6 wt.% induced no significant negative influences on the transverse strength and elastic modulus of PMMA dental resin (Att et al. 2009), more studies needs to be performed to investigate the influences of NAC on other important mechanical properties, such as degree of conversion (DC), microhardness, surface roughness, of PMMA dental resins.

73   Thus, the purposes of the present study are: first, to confirm the protective effects of NAC against

74   the cytotoxicity of PMMA dental resin; second and more importantly, to investigate the influences of

75   NAC on various mechanical and physical properties of PMMA dental resin.

76 **Materials and methods**

77

78 **Resin preparation**

79 Self-polymerizing PMMA resin (Unifast Trad, GC, Tokyo, Japan) was prepared by mixing the powder

80 and liquid components for 30 s in accordance with the manufacturer's recommendations (powder/liquid

81 ratio of 1.0 g/0.5 mL) in a 12-well culture plate. NAC (Sigma; St Louis, MO, USA) was prepared as

82 1.0 mol/L stock solution in HEPES buffer (Sigma; St Louis, MO, USA), and the pH was adjusted to

83 7.2 as previously reported (Yamada et al. 2008). To prepare NAC-loaded PMMA resin (0.15, 0.3, 0.6,

84 0.9 wt%), 13.8, 27.6, 55.2 or 82.8 μL NAC solution was added to the liquid MMA to a final volume of

85 0.5 mL, and then this NAC-incorporating liquid was mixed with 1.0 g powder. After 30 min of setting,

86 the polymerized resin block was rinsed with ddH$_2$O once.

87

88 **Preparation of the extract**

89 The extract of the polymerized resin was prepared by soaking PMMA resin specimens with or without

90 NAC in alpha-modified Eagle's medium (α-MEM; Gibco BRL Division of Invitrogen, Gaithersburg,

91 MD, USA) without serum in the 12-well culture plate at 37°C in a humid atmosphere of 5% CO$_2$ for 24

92 h. According to ISO 10993-12:2007, the ratio between the surface of the sample and the volume of the

93 added medium was 1.25 cm^2/mL. The eluent was filtered for sterilization and 10% fetal bovine serum

94 (Gibco) was added. The extract was stored at 4°C before use.

95

96 **Quantification of released NAC with high performance liquid chromatography (HPLC)**

97 HPLC analysis was performed to determine the concentration of released NAC from the experimental

98 PMMA resin containing 0.9 wt% of NAC. The extract was prepared as previously described. HPLC

99 analysis (Model Shimadzu LC-2010A, Shimadzu Corporation, Kyoto, Japan) was performed at 220 nm

100 on an InertSustain C18 column (5 μm, 4.6 mm×250 mm, GL Sciences). The column was maintained at

101 50°C and the samples were eluted for 5 min. The mobile phase, at a flow rate of 0.7 mL/min, consisted

102 of methanol and 0.2 % phosphoric acid with 100 mM sodium perchlorate. The volume of sample

103 injected was 20 μL. Identification of the analyte, NAC, was made based on the retention time of the

104 NAC peaks registered for the standard solutions. Standard curve was drawn using NAC solution with

105 various concentrations (1, 5, 20, 40, 60, 80 and 100 μg/mL). After the linearity of the standard curve

106 was confirmed by linear regression analysis, the concentration of released NAC from the experimental

107   PMMA resin was calculated using the standard curve. The experiments were repeated for three times,

108   and the mean value was determined as the concentration of released NAC.

109

Cell cultures

111   Human dental pulp cells (HDPCs) were isolated from dental pulp tissue of non-carious third molars

112   extracted from young healthy patients (18-25 years old) according to a protocol approved by the Ethics

113   Committee of the Fourth Military Medical University. Briefly, the pulp was harvested, minced and

114   digested in a solution containing 3 mg/mL type I collagenase and 4 mg/mL dispase (Gibco,

115   Gaithersburg, MD, USA) at 37°C for 2 h (Hilkens et al. 2013). Single-cell suspensions were obtained

116   by passing the cells through a 70-μm strainer (BD Falcon, Franklin Lakes, NJ, USA). The cells were

117   cultured in α-MEM (Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 units/mL

118   penicillin and 100 mg/mL streptomycin. The medium was changed every 3 days. Cells from the second

119   passage were used for both cell attachment assay and cytotoxicity assays.

120

Cell viability test

122   Mitochondrial dehydrogenase activity was measured by MTT assay to investigate cell viability.

123   HDPCs were seeded into 96-well culture plates at a density of 5×10^3 cells/well and incubated at 37°C

124   and 5 % CO_2 for approximately 24 h. When the cells grew to 80% confluence, the medium was

125   replaced by the extract of PMMA resin with or without NAC. After each incubation period (3 days,

126   and 7 days), 20 μL of MTT solution at 5 mg/mL (Sigma-Aldrich, St. Louis, MO, USA), disinfected by

127   infiltration, was added to the well, and the plates were incubated for a further 4 h. After disposal of the

128   culture medium, 200 μL dimethyl sulfoxide (DMSO) (Amresco, Solon, OH, USA) was added to each

129   well. After gently swirled for 10 min, the absorbance of each well at 490 nm was measured. The blank

130   group contains only complete culture medium without cells. For the control group, cells were cultured

131   in wells without eluent. Relative cell viability was calculated using the following equation:

132   Relative cell viability = ($OD_{experimental}$ - OD_{blank}) / ($OD_{control}$ - OD_{blank})

133   The experiments were repeated for three times. Cytotoxicity was rated in accordance with ISO-

134   standard 10993-5 as non-cytotoxic (cell viability higher than 75%), slightly cytotoxic (cell viability

135   ranging from 50% to 75%), moderately cytotoxic (cell viability ranging from 25% to 50%), and

136   severely cytotoxic (cell viability lower than 25%).

137   The MTT assay was also employed to evaluate the influence of NAC alone on cells. Using HPLC

138   analysis, we confirmed that the concentration of NAC released from the experimental PMMA resin

139   containing NAC was no higher than 0.54 mM. Thus, to exclude the possibility that released NAC may

140   enhance the proliferation of cells, we used 0.54 mM NAC alone to treat the cells and studied its

141   influence on cell viability with MTT assay.

142

143   **Cell attachment assay**

144   Disc-shaped specimens (10-mm in diameter and 2-mm in thickness) of each subgroup were fabricated

145   using a mould. The paste was filled in the mould and covered with a glass silde. All the specimens

146   were allowed to set for 30 min at 25°C. All discs were rinsed with ddH_2O once and sterilized by

147   ethylene oxide gas before use. HDPCs were seeded onto the discs at the density of 2.0×10^6 cells/well

148   in a 6-well culture plate. After incubation for 24 h, the samples were rinsed with phosphate-buffered

149   saline (PBS, pH 7.2) to eliminate unattached cells and fixed with 2.5% glutaraldehyde and 2%

150   paraformaldehyde in 0.2 M sodium cacodylate buffer (pH=7.4). After being freeze-dried and sputter-

151   coated with platinum (E-1030; Hitachi, Tokyo, Japan), the specimens were observed with SEM (Model

152   S-4800; Hitachi, Tokyo, Japan) to examine cell morphology and attachment.

153

154   **Measurements of degree-of-conversion (DC)**

155   The degree of auto-polymerization conversion of control and experimental resin specimens was

156   measured by Fourier Transform Infrared Spectroscopy (FTIR 8400S, Shimadzu Scientific Instruments,

157   Kyoto, Japan). The resin paste was placed between two polyethylene films and pressed to form a very

158   thin film. The FTIR spectra were collected after 5 min, 10 min, 20 min, 30 min, 1 h, 4 h and 24 h.

159   FTIR spectrum of the uncured mixture was also recorded as reference. For each subgroup, the

160   measurement was repeated for three times.

161   DC of the resin specimens were calculated using the following equation:

162    $$DC(\%) = \left[1 - \frac{(Abs_{ana}/Abs_{ref})_{cured}}{(Abs_{ana}/Abs_{ref})_{uncured}} \right] \times 100\%$$

163   Abs_{ana}: the intensity of the carbon-carbon double bond stretching vibration (peak height at 1638 cm^{-1});

164   Abs_{ref}: the intensity of the symmetric ring stretching at 1610 cm^{-1};

165   Absorbance peak intensity values on the FTIR spectra were calculated using OMNIC 8.0 software

166   (Spectra Tech, USA).

167

Flexural strength

Flexural strength was tested according to ISO 4049:2009 standard. PMMA resin specimens (2 mm × 2 mm × 25 mm, 12 specimens for each subgroup) were prepared in a rectangular stainless steel mold. After 24 h of setting, the specimens were removed from the mold. The three-point bending test was performed using a universal testing machine (AGS-10kNG, Shimadzu, Kyoto, Japan) at a cross-head speed of 1 mm/min, at a controlled room temperature. The flexural strength (FS) was calculated by the equations:

$$FS = \frac{3PL}{2wh^2}$$

where P is the maximum load exerted on the specimen (N), L is the distance between the supports (25 mm), w and h are, respectively, the width (2 mm) and the thickness of the specimen (2 mm). Mean flexural strengths were calculated in MPa (megapascals). Three specimens were tested for each group. After testing, the microstructure of the fractured surfaces obtained from mechanical test were observed with SEM.

Microhardness

Microhardness test (Vickers test) was performed using the specimens after the three point bending test. Five half-bars from each subgroup were tested. Vickers microhardness was measured using a microhardness tester (HX-1000TM, Taiming, Shanghai, China). The indenter point was kept on the surface for 35 s with 25 g load. Three indentations were made on each specimen.

Surface roughness

Three half-bars from each subgroup tested by flexural strength testing were randomly chosen for analysis of surface roughness. The upper and lower surfaces of the specimens were polished with SiC grinding paper (320-grit, 600-grit, 1200-grit and 2000-grit) under running water on a polishing machine (UNIPOL-830, Shenyang Kejing Automation, Kejing, China). Specimens were ultrasonically (Vitasonic II, VITA Zahnfabrik, Berlin, Germany) cleaned for 10 min before testing. The surface roughness was analyzed with a 3D non-contact optical profilometer (PS50 Optical Profilometer, Nanovea, Irvine, CA). This device allowed a scanning over an area of 2 mm in length (x-axis) and 2 mm in width (y-axis). Surface roughness (Ra) was calculated for each scanned area.

197

Statistical analysis

199 The results for cell viability, degree of conversion, flexural strength, microhardness and surface
200 roughness were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test.
201 SPSS 16.0 software (SPSS Inc, Chicago, IL, USA) was used for the statistical analysis. Statistical
202 significance was preset at $p=0.05$ for all tests.

Results

Quantification of NAC elution using HPLC

Figure 1 shows representative chromatograms for NAC standard solution (0.1 mg/mL) and eluted NAC from experimental specimen containing 0.9 wt% of NAC. Identification of the analyte, NAC, was made based on the retention time of the NAC peaks registered for the standard solutions. The relationship obtained for the linear peak area (A) and concentration (c) for the analyte (NAC) was: $A=27053c+48133$, with a correlation coefficient $R^2=0.99859$. According to the calibration curve, the concentration of NAC released from the experimental resin containing 0.9 wt% of NAC was calculated as 0.54 ± 0.02 mM.

MTT assay

MTT assay revealed that, as compared to control group, cell viability at day 3 and day 7 was remarkably lower in the culture with PMMA extract (**Fig. 2A**). NAC exhibited protective effects on the cytotoxicity of PMMA in a concentration-dependent manner. Cultures with the extract of PMMA incorporating NAC showed higher cell viability than untreated PMMA resin. At day 7, relative cell viability was 77% for PMMA resin containing 0.15 wt.% NAC, which is more than 10-fold of that in subgroup 0 wt% ($p < 0.05$) (**Fig. 2A**). PMMA resin containing 0.9 wt% NAC presented the highest relative cell viability, reaching 95.6 % at day 3 and 97.6 % at days 7 (**Fig. 2A**). **Figure 2B** showed that 0.54 mM of NAC alone, based on results of HPLC, induced no significant influence on the viability of HDPCs ($p > 0.05$).

Cell attachment assay

Figure 2C shows the attachment of HDPCs on the surfaces of specimens at day one. In subgroup 0 wt% and subgroup 0.15 wt%, cells grew poorly. Cells with round or collapsed appearances were observed in these subgroups. In subgroup 0.3 wt%, 0.6 wt% and 0.9 wt%, HDPCs attached and spreaded well on the specimens, exhibiting spindle- or polygonal-shapes. The number of adhering cells increased as the concentration of NAC increased in the experimental PMMA resins. The surface of subgroup 0.9 wt% was almost fully covered by HDPC cells.

Degree of conversion

234   DC was measured from 5 min to 24 h after the liquid MMA was mixed with PMMA powder (**Fig. 3**).

235   During the first 10 min curing period, DC increased dramatically. At 10 min, the DCs of the control

236   and experimental subgroups ranged from 41.0 % to 58.8%. Experimental subgroups containing NAC

237   showed significant lower DC values as compared to control subgroup ($p < 0.05$). After 10 min, DC

238   increased in a relatively slow manner. At 24 h, the DC values of the five experimental subgroups were

239   all higher than 72.0%. In particular, the DC for experimental PMMA resin containing 0.15 wt% NAC

240   reached 75.2 % after 24 h of setting, showing no significant difference to the control PMMA resin

241   without NAC ($p > 0.05$). Whereas, experimental PMMA resin with 0.3 wt% or higher concentration of

242   NAC revealed slightly but statistically compromised DC values.

243

244   **Flexural strength testing**

245   The flexural strength (FS) of specimens in various subgroups after 24 h of setting are shown in **Fig. 4A**.

246   Adding NAC to PMMA resin induced a negative influence on the flexural strength of the material.

247   Although the experimental resin containing 0.15 wt% NAC showed similar flexural strength (91.0±6.2

248   MPa) to that of control group (95.9±5.8 MPa), subgroups with higher concentration of NAC exhibited

249   significantly compromised flexural strength. And the PMMA with 0.9 wt% showed the lowest flexural

250   strength of 72.8 MPa ($p < 0.05$).

251

252   **Microhardness**

253   **Figure 4B** showed the results of microhardness test after 24 h of setting. The trend of surface

254   microhardness was similar to that of flexural strength. The incorporation of 0.15 wt% NAC had no

255   significantly adverse effects on the microhardness of PMMA resin ($p>0.05$ as compared to control

256   group without NAC). However, when the concentration of incorporated NAC was further increased,

257   the microhardness of the materials showed a significantly reduced value.

258

259   **Surface roughness**

260   **Figure 4C** show the mean Ra values of the specimens. The mean Ra values was 0.60 μm and 0.86 μm

261   for subgroup 0.15 wt% and 0.3 wt%, respectively, which is not significantly different from that of

262   control group. However, other subgroups with higher concentrations of NAC revealed significantly

263   higher Ra values, indicating that adding NAC beyond the concentration of 0.3 % has negative

264   influences on the surface roughness of PMMA resin.

265

266 **SEM of fractured surfaces**

267 The SEM image of the fractured surfaces for each subgroup is presented in **Fig. 4D**. As can be seen,

268 PMMA resin without NAC showed a relatively smooth appearance. The fractured surface of

269 experimental resin containing 0.15 wt% NAC was similar to that of control group. However, for the

270 subgroup with 0.3 wt% or higher concentration of NAC, pit-like internal defects can be clearly seen.

271 Higher concentration of NAC incorporation is accompanied with greater number of internal defects.

272

Discussion

273

274

275 This study investigated the biocompatibility and mechanical properties of PMMA resin containing

276 different concentrations of NAC. It was found that adding NAC to PMMA resin could reduce its

277 cytotoxicity. However, the concentration of NAC should not exceed 0.15 wt% because too much NAC

278 would compromise the mechanical and physical properties of the PMMA resin.

279   MTT study revealed that the exposure of HDPCs to eluent of PMMA resin resulted in an

280 overwhelming cell death. Relative cell viability of HDPC cultured with eluent of PMMA resin was

281 reduced to 12 % at day 3 and 7.6 % at day 7, indicating that this material is severely cytotoxic. This

282 result is in accordance with previous studies (Att et al. 2009; Kojima et al. 2008; Tsukimura et al. 2009;

283 Yamada et al. 2009; Yamada et al. 2008). It is well accepted that polymerization of resinous dental

284 materials is never complete (Kopperud et al. 2011; Miletic et al. 2009) and the unpolymerized

285 monomers can be released over time. The unpolymerized monomers can be the primary reason for the

286 cytotoxicity of resinous dental materials (Att et al. 2009). In case of PMMA resin, its cytotoxicity can

287 be attributed to the release of unpolymerized MMA monomer. Similar to other methacylate monomers,

288 MMA can disturb the intracellular redox balance and thus induce negative influences on the function

289 and viability of cells (Att et al. 2009; Ciapetti et al. 2002; Huang & Chang 2005; Ratanasathien et al.

290 1995; Vale et al. 1997).

291   NAC, a well-known antioxidant, has been recognized as an effective agent that can reduce the

292 cytotoxicity of dental monomers and thus improve the biocompatibility of resinous dental materials

293 (Gillissen et al. 1996; Ma et al. 2013; Nocca et al. 2010; Paranjpe et al. 2007a; Paranjpe et al. 2007b;

294 Paranjpe et al. 2009; Spagnuolo et al. 2013; Yamada et al. 2008; Zafarullah et al. 2003). In our study, it

295 was found that incorporation of NAC to PMMA resin significantly salvaged HDPC from resin-induced

296 cell death. When added at the concentration of 0.15 wt%, NAC remarkably increased relative cell

297 viability by almost 10 times at day 7 (7.6 % for control PMMA resin without NAC versus 77 % for

298 experimental resin containing 0.15 wt% NAC). This result is similar to that of Wael's study, which

299 found that cell density was significantly higher on NAC-supplemented resin compared to that on the

300   untreated resin at days 2 and 5 (Att et al. 2009). To rule out the possibility that the improved cell

301   viability related to NAC-containing PMMA resin is due to the pro-proliferating effects of NAC itself,

302   we quantified the concentration of NAC released from the experimental PMMA resin containing 0.9

303   wt.% NAC and studied the influence of NAC *perse* on the proliferation of HDPC. According to the

304   result of HPLC study, the concentration of NAC released was 0.54 mM. When 0.54 mM of NAC alone

305   was used to treat the cells, no significant pro-proliferating effect was observed. To further prove the

306   protective effect of NAC against PMMA-related cytotoxicity, we observed cell attachment on

307   specimens from various subgroups. In accordance with the results of MTT study, SEM observation

308   revealed that cell attachment and growth on PMMA resin containing NAC was remarkably enhanced

309   as compared to the control group without NAC. Altogether, the results of MTT study and SEM

310   observation indicate that incorporation of NAC can be an effective strategy to improve the

311   biocompatibility of PMMA resin.

312   Aside from biocompatibility, the clinical success of the auto-polymerizing PMMA materials also

313   depends on their mechanical properties (Balkenhol et al. 2008; Burke et al. ; Ferracane & Greener 1986;

314   Lovell et al. 2001; Lujan-Climent et al. 2008; van der Bilt et al. 2008). Thus, we investigated the

315   influence of NAC incorporation on DC, flexural strength, microhardness and surface roughness of

316   PMMA resin.

317   FTIR confirmed that the polymerization of PMMA resin with or without NAC was a time-

318   associated reaction. Within the first ten minutes after mixing, the specimens polymerized at a relatively

319   fast rate and the DC reached a high level after this period. This result is in agreement with previous

320   studies, which found that the greatest increase of DC happens within the first 10 min after mixing of

321   dental resins (Balkenhol et al. 2007; Ferracane 1985). In our study, DC for the control group without

322   NAC was as high as 58.8% after 10 min of setting. Incorporation of NAC reduced DC in a dose-

323   dependent manner, with the subgroup containing 0.9 wt.% NAC presenting the lowest DC of

324   approximately 41.0 %. In the following period from 10 min to 24 h, the polymerization process of all

325   subgroups slowed down and kept at an almost fixed rate. At 24 h, DC for all the tested subgroups was

326   above 70 %. While the experimental resin containing 0.15 wt.% NAC showed a DC value that is not

327 significantly different from that of control, those incorporating higher concentration of NAC presented

328 a significantly lower DC. Such reduced degree of conversion related to NAC-incorporating PMMA

329 resin might be attributed to the creation of a heterogeneous system with reduced local concentration of

330 MMA by unpolyerizable NAC.

331   Although DC is not the only factor that determines the mechanical properties of dental polymers,

332 lower DC is usually associated with poorer mechanical properties. In our study, it was found that

333 incorporation of NAC at concentrations higher than 0.15 wt.% significantly reduced flexural strength

334 and microhardness of the PMMA resin. Observation of the fractured surface revealed that adding too

335 much NAC produced defects in PMMA resin specimens. Such defects may represent voids that are left

336 after the release of NAC or unpolymerized MMA. When such voids present on the outer surface of the

337 material, rough surfaces may be produced. This explains the higher surface Ra values of resin

338 specimens containing high concentration of NAC.

339   Although too much NAC imposed negative influences on flexural strength, microhardness and

340 surface roughness of the carrier material, limiting the concentration of NAC to 0.15 wt% can be an

341 effective strategy to generate experimental PMMA resin with acceptable mechanical properties. As for

342 flexural strength, the measured results of PMMA incorporating 0.15 wt.% and 0.30 wt.% NAC were

343 91.0 MPa and 83.3 MPa, respectively, which all exceeded the minimum values stipulated by the ISO

344 flexural strength standard of 80 MPa for self-curing dental polymer-based restorative materials.

345 Although there is no specific requirement for microhardness in the ISO standard, the value of our

346 experimental PMMA resin containing 0.15 wt.% NAC was comparable to other commercial resin

347 materials for temporary restorations (Jo et al. 2011). As for surface roughness, the Ra value for 0.15

348 wt.% NAC-containing PMMA resin was 0.6. According to some studies, surfaces with Ra values lower

349 than 0.75 μm can be classified as smooth surfaces (Busscher et al. 1984; Quirynen et al. 1990).

350 Moreover, the Ra value of 0.6 μm for PMMA resin containing 0.15 wt.% NAC is close to the

351 recommended surface roughness threshold (0.2 μm) to minimize plaque accumulation (Bollenl et al.

352 1997; Quirynen et al. 1990; Ulusoy et al. 1986; Verran & Maryan 1997).

353   To conclude, incorporation of NAC reduced the cytotoxicity of dental PMMA resin. Although

354   adding too much NAC to PMMA resin resulted in compromised DC, flexural strength, microhardness

355   and surface roughness, limiting the concentration of NAC within 0.15 wt.% remarkably improved

356   biocompatibility of PMMA resin without exerting significant negative influence on mechanical

357   properties. Therefore, incorporation of NAC should be considered as an effective strategy to produce

358   clinically   reliable   and   biocompatible   PMMA   dental   resins.

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Fig. 1 Typical HPLC chromatogram of standard solution of NAC (0.1 mg mL^{-1}) and released NAC from experimental PMMA resin containing 0.9 wt% of NAC.

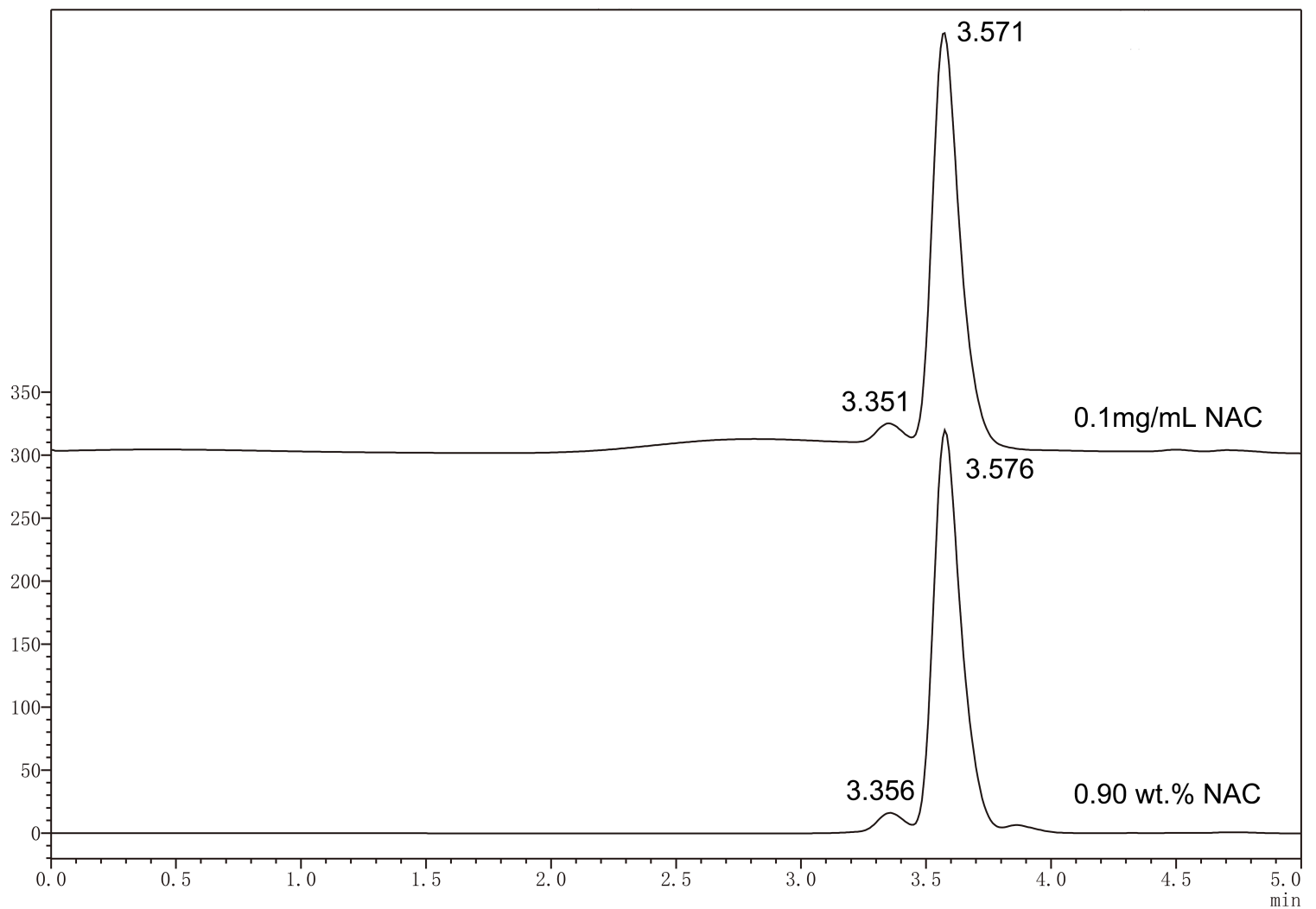
Identification of NAC was made based on the retention time of the NAC peaks registered for the standard solutions.

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Fig. 2 Cytotoxicity of PMMA resin with or without NAC.

Data are presented as the mean±SD of three independent experiments performed in quintuplicates. (A) Effects of the extract of PMMA resin with or without NAC on the viability of HDPCs at day 3 and day 7. For each tested time period, values with different superscripts are significantly different from each other (One-way ANOVA, $p<0.05$). (B) Effects of 0.54 mM NAC on the viability of HDPCs at at day 3 and day 7. Values with different superscripts are significantly different from each other (One-way ANOVA, $p<0.05$). NS: not significant between the control group and the experimental group. (C) Typical SEM pictures showing cell attachment and morphology on top of PMMA resins with or without NAC. The cells with round or collapsed appearances were observed in subgroups containing no or 0.15 wt.% NAC (arrows).
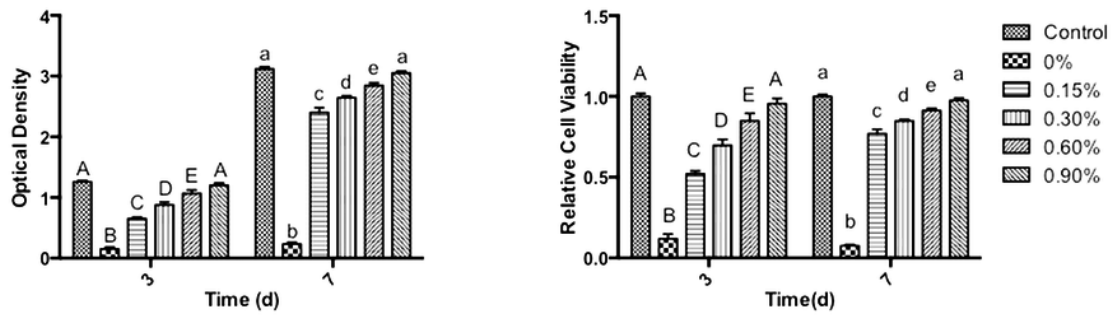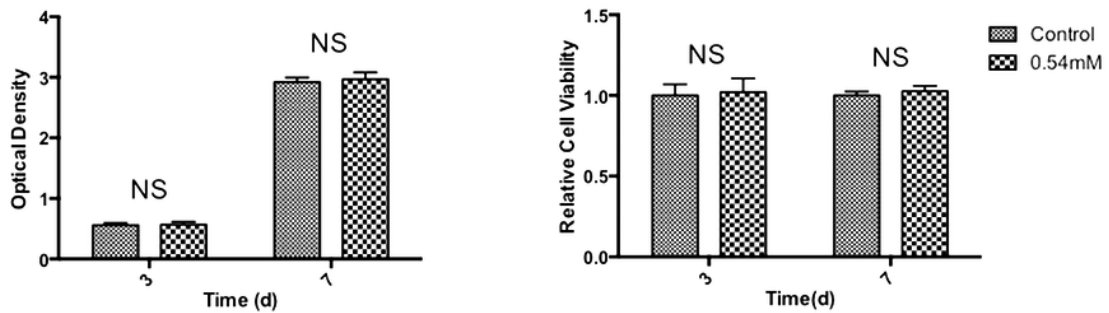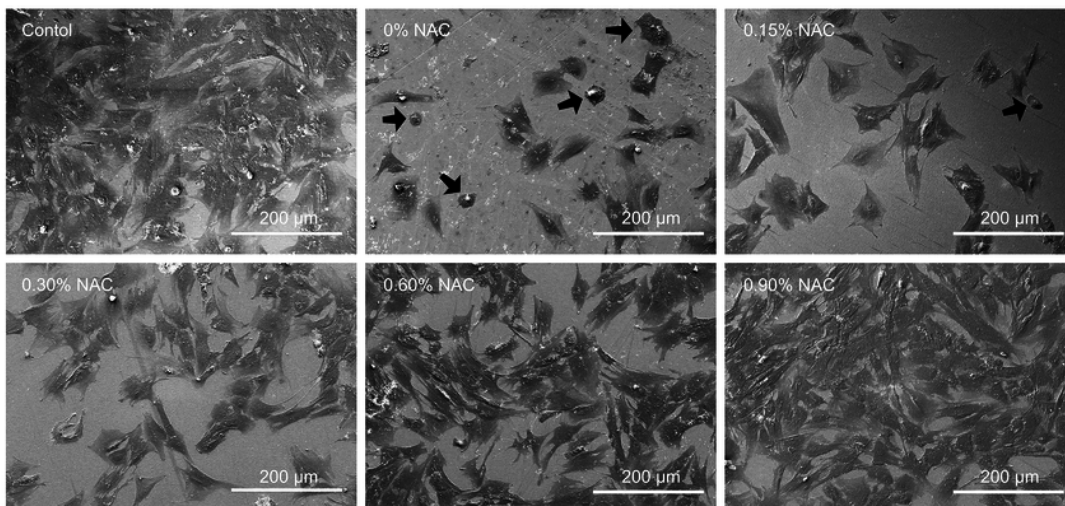
Fig. 3 Degree of conversion of PMMA resin with different concentrations of NAC at specific time after mixing.

Results are presented as mean±SD of three independent experiments.
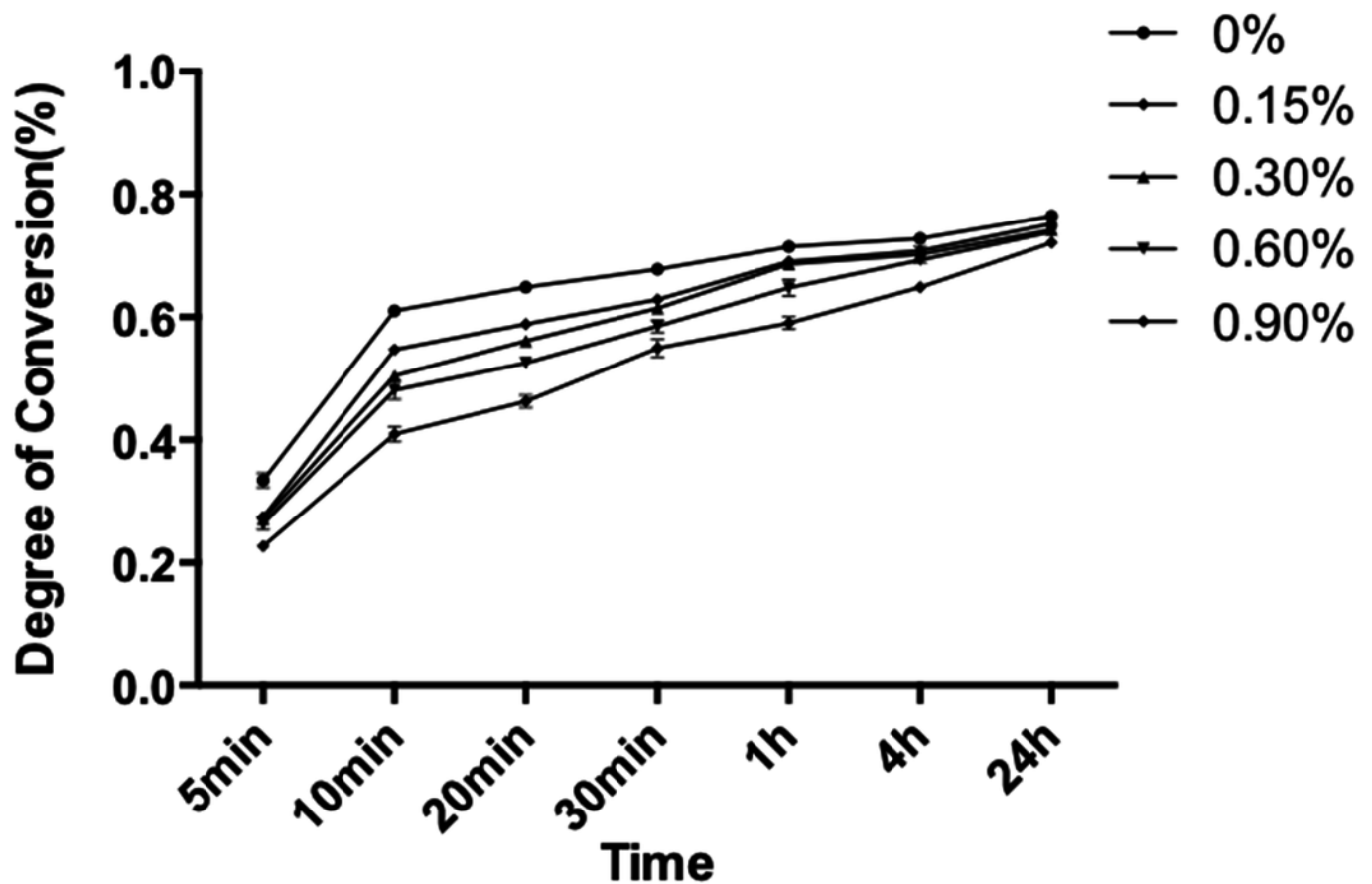
Fig. 4 Mechanical properties of PMMA resin with or without NAC.

Data are presented as the mean±SD. Values with different superscripts are significantly different from each other (One-way ANOVA, p<0.05). (A) The flexural strength (FS) of specimens in various subgroups after 24 h (n=12). (B) Microhardness value (VHN) after 24 h (n=15). (C) Surface roughness (Ra) of the specimens (n=3). (D) The SEM image of the fractured surfaces for each subgroup. Pit-like internal defects can be observed for subgroup with NAC (arrows).