

In vitro bleaching study with a 20 % hydrogen-peroxide system

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Fig. 1a

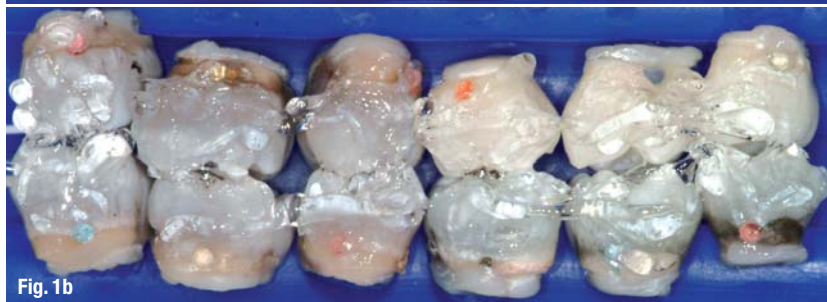


Fig. 1b

Figs. 1a & b Stained teeth before and after application of the 20 % whitening gel.

While nightguard vital bleaching is the most used treatment modality for whitening of discoloured teeth, in-office tooth whitening procedures that use a concentration of higher than 10 % carbamide peroxide have been advocated as acceptable alternatives. Patients often choose in-office bleaching when they desire more rapid whitening of their teeth and/or when they do not desire to wear bleaching trays for at-home techniques.¹ Although manufacturers indicate that most in-office bleaching agents can be used without light irradiation, studies have reported that light activation can enhance the results of the bleaching procedures.² The light energy increases the rate of

hydrogen peroxide degradation to form oxygen free radicals, leading to an enhancement of the bleaching process.²⁻⁵

Introduced in 1948, tetracyclines (TCN) are broad-spectrum antibiotics that may be used in the treatment of many common infections in children and adults. One of the negative side-effects of TCN is incorporation into tissues that are calcifying at the time of drug administration, such as teeth, cartilage and bone. A resulting permanent discolouration of both the primary and permanent dentitions can vary, such as yellow or grey or brown, depending on the dosage and the type of the drug received, as well as the duration of exposure to the drug.^{6,7}

In recent literature, it has been noted that Minocycline, a semi-synthetic member of the TCN family widely used in the treatment of acne, also causes abnormal pigmentation of collagenous tissues such as the skin, thyroid gland, nails, bone, sclera and conjunctiva in adults. The pigment is the product of an oxidation reaction, and laboratory studies have shown that the pigment formation can be induced by exposure to ultraviolet light in the presence of air.⁸ Similarly, Minocycline has been shown to cause a blue-grey to grey discolouration of the teeth of adults. This appears to be linked to the cycle of demineralisation and remineralisation of the enamel surface, and can produce staining that is clinically indistinguishable from staining during tooth development.⁹

As a continuation of what was reported by Harada¹⁰, we recently described an effective *in vitro* method to produce standardised discolouration of extracted human molar teeth using Chlortetracycline and its analogs.¹¹ Utilising this technique, a readily available source of discoloured teeth can be produced to evaluate the efficacy of various bleaching protocols.

The purpose of the present study was to evaluate the effects of one in-office tooth whitening procedure (Zoom 2, Discus Dental) on such standardised discoloured teeth to assess their usefulness as models for bleaching research. Our hypothesis was that the teeth discoloured by the *in vitro* technique

Fig. 2 Stained teeth demonstrating application of the mercury metal halide light. Note the way that all the stained teeth fit inside the scope of the light.

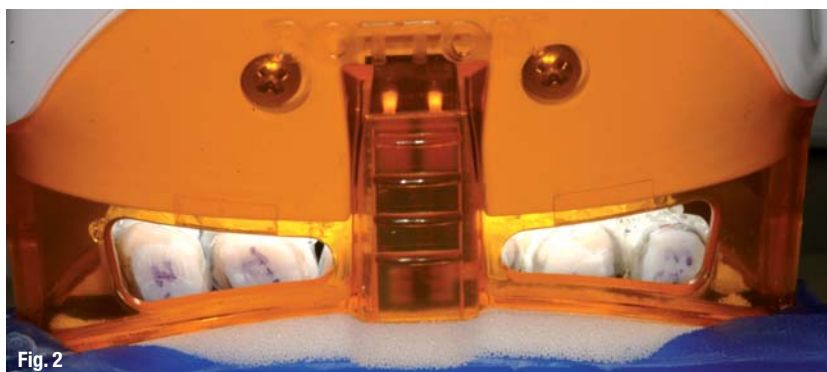


Fig. 2

would whiten in the same manner and to the same degree as non-experimental teeth.

Materials and methods

Twenty extracted human molar teeth were sectioned approximately 2 mm above their furcations to provide the clinical crowns for this experiment. Images of the buccal and lingual surfaces were recorded with a digital camera (Canon EOS-D30, Canon) using standardised settings throughout the experiment. Each tooth was then analysed with a dental spectrophotometer (VITA Easyshade, VITA) against a neutral grey background.

After baseline recordings, the pulp chambers were etched for 60 seconds with 37 % phosphoric acid gel and then thoroughly rinsed with distilled water. Saturated solutions of Chlortetracycline, Doxycycline and Minocycline were produced by mixing 100 to 250 mg of the five respective medications in 5 ml of distilled water. Only the resulting supernatant was recovered for use. Five pulp chambers were filled with 0.5 ml of each of the solutions. Distilled water was used as a control in the five remaining pulp chambers. Composite resin disks were used to seal the chambers of all teeth.

The teeth were then placed crown first into a centrifuge tube and subjected to centrifugation at 2,800 rpm for 20 minutes. After centrifugation, the teeth were stored in distilled water for one week before exposure to continuous light irradiation with two 60 W Xenon lamps for seven weeks. Digital images of the colour change of both the buccal (B) and lingual (L) surfaces were recorded against a neutral grey background. The chroma (C*), brightness (L*) and hue (H*) values were recorded with the spectrophotometer, and the colour differences (ΔE) between baseline and seven weeks were calculated from the spectral data. Colour differences were calculated using CIE ΔE*, using the following formula:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta C^*)^2 + (\Delta H^*)^2]^{1/2}$$

In-office whitening procedure

At the completion of the discolouration phase, three randomly selected samples from each group were subjected to Zoom 2 in-office tooth whitening procedures. The Zoom 2 system uses a hydrogen-peroxide-based tooth whitener, which comes pre-packaged as two individual components: a 25 % hydrogen peroxide gel and a proprietary activator. These two components are mixed at room temperature to form a working gel that has a 20 % concentration of hydrogen peroxide and a pH between 7.5 and 8.5 (Figs. 1a & b). Prior to the application of

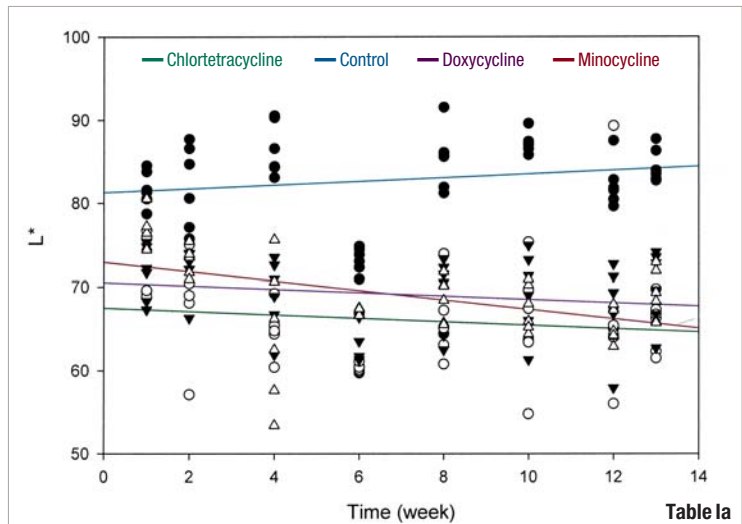


Table 1a

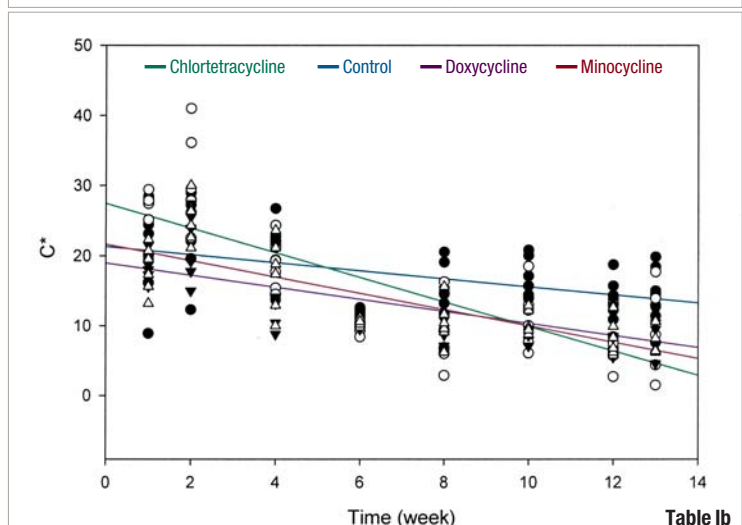


Table 1b

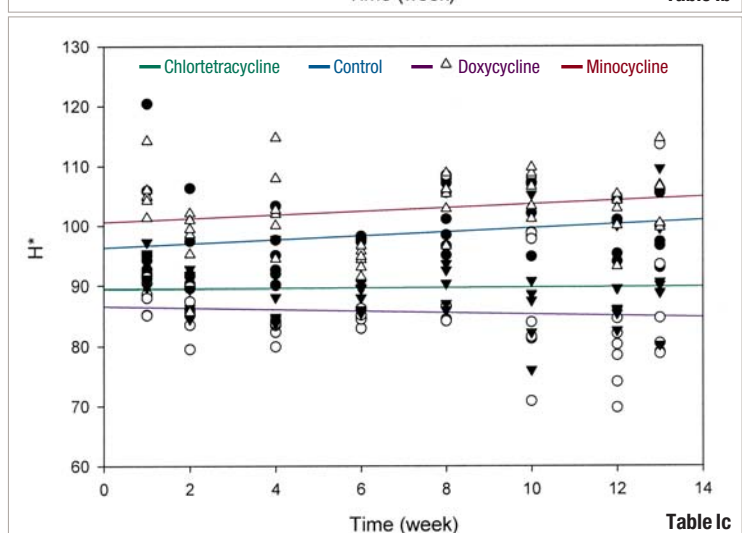


Table 1c

the gel to the sample teeth, the samples were coated with a solution supplied by the manufacturer in the form of pre-treatment starter swabs.

Tables 1a-c Regression plots of L*, C* and H*.

The treatment sessions were broken down into three 15-minute applications of the 20 % concentration hydrogen peroxide gel. Between each

Figs. 3a–d The stained teeth and control groups demonstrating progress from baseline through weeks 1, 3, 5 and 7.

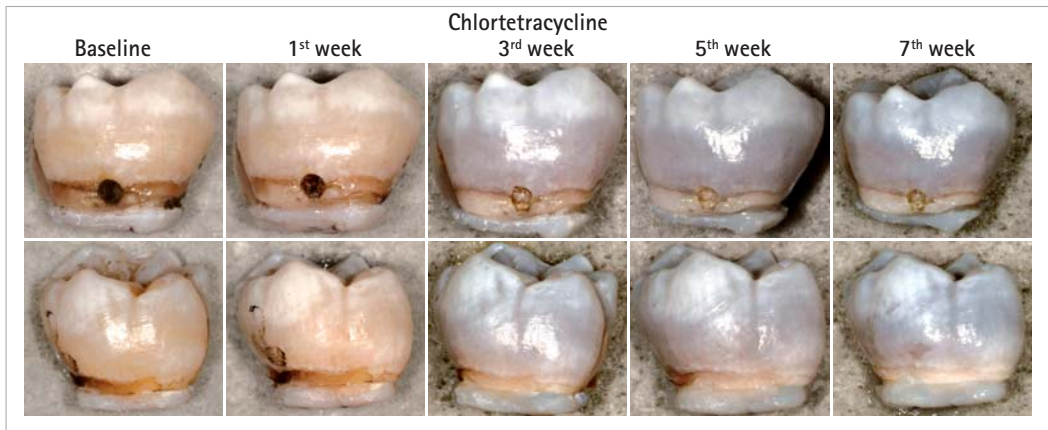


Fig. 3a

application, the gel was removed with high volume suction and the samples were wiped clean with cotton gauze saturated in distilled water. Therefore, the total exposure of the teeth to the bleaching agent was limited to 45 minutes per session. Sessions were performed once a week for seven weeks.

The gel applications were activated by the use of the Zoom 2 mercury metal halide light unit that, according to the manufacturer, emits violet coloured light with a wavelength in the range of 350 to 400 nm. When placed into position, the light unit is capable of illuminating all of the teeth simultaneously (Fig. 2). The use of this light unit implies that a specific wavelength of light activates some aspect of the whitener and enhances the whitening effect. An infrared filter helps to minimise the amount of heat generated at the surface of the teeth during the treatment session.

Immediately following the completion of each 45-minute session, digital images of both the buccal and lingual surfaces were recorded against a neutral grey background. The L*, C*, and H* values were also recorded with the spectrophotometer. The samples were stored in distilled water until the next session to prevent desiccation.

Results

The means of the L*, C* and H* values for each of the TCN derivatives and the control as recorded by the spectrophotometer at the specified weekly intervals are listed in Table II. Tables Ia to c show the regression plots of the L*, C* and H* values for the TCN and control groups. Statistical analysis of the changes in L*, C* and H* between baseline and the final evaluation was performed. There was a significant association amongst treatment group, evaluation and interaction term, treatment group x evaluation, and L* (two-way ANOVA; $p < 0.001$, $p = 0.002$ and $p < 0.001$, respectively). All groups except Doxycycline demonstrated statistically significant differences in brightness between the baseline and the final evaluation (Holm-Sidak Multiple Comparisons Test; all comparisons to baseline; $p < 0.05$). For the Chlortetracycline-, Doxycycline- and Minocycline-stained groups a decrease in brightness rather than an increase was observed. The control group demonstrated a significant increase in brightness (Table Ia).

The mean L* for the control group was significantly higher than the Chlortetracycline, Doxycycline and Minocycline groups at baseline. Since

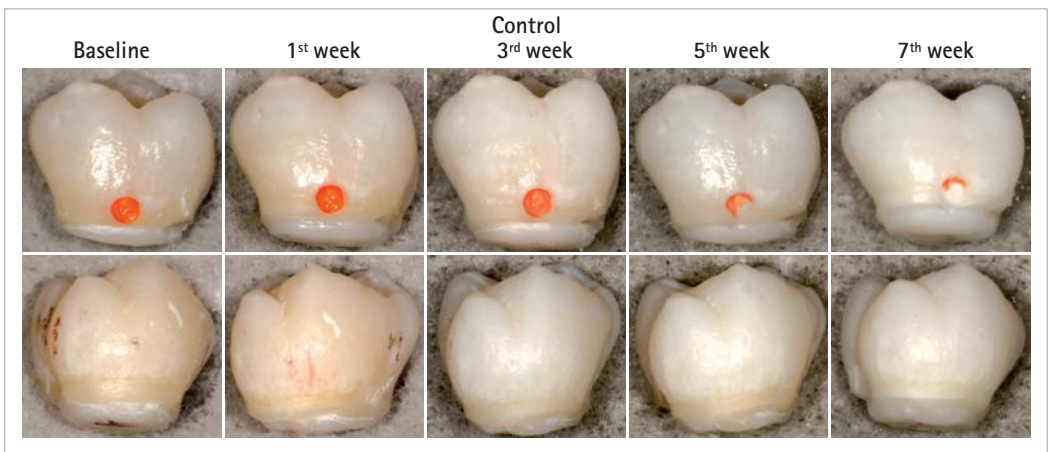


Fig. 3b

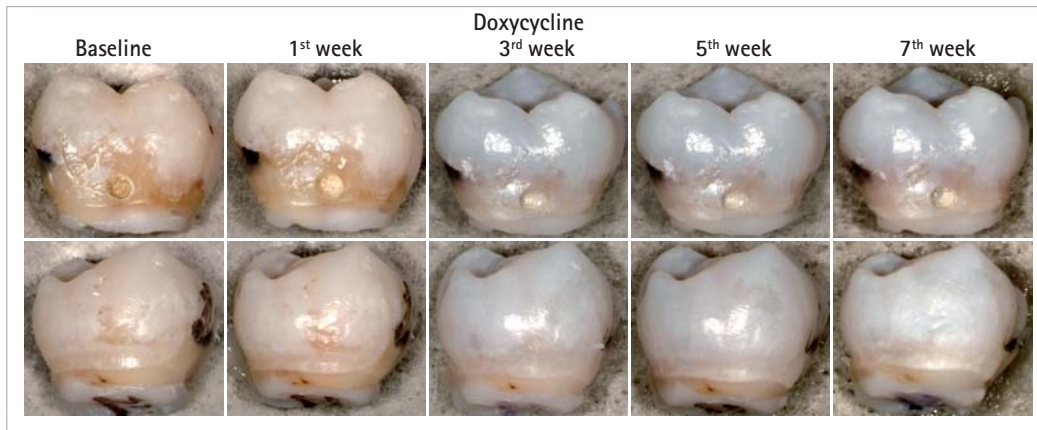


Fig. 3c

the mean L^* for the control group increased significantly between baseline and final measurements and the Chlortetracycline, Doxycycline and Minocycline groups' mean L^* decreased, these differences were even greater at the final evaluation period (Table Ia). While these changes explain the significant differences between the four groups, they do not indicate a positive whitening effect.

There was a significant association between C^* and treatment group, evaluation and the interaction term, treatment group x evaluation (two-way ANOVA; $p < 0.003$, $p < 0.001$ and $p = 0.002$, respectively). From the baseline to final evaluation, all four groups demonstrated a significant reduction in C^* (Holm-Sidak Multiple Comparisons Test; all comparisons to baseline; $p < 0.05$; Table Ib). With the C^* data, we once again see that there was a significant difference between the control group and the Chlortetracycline group at baseline, with the Chlortetracycline group significantly more chromatic.

At the final evaluation, the reduction in C^* for the Chlortetracycline, Minocycline and Doxycycline groups was so dramatic that the control group

was significantly more chromatic than these three groups (Holm-Sidak Multiple Comparisons Test; $p < 0.05$). There was no significant association between H^* data and the evaluation period (Table Ic). There was a significant association between treatment group and H^* . Comparing the means for the three experimental groups to the control, the hue value for Chlortetracycline was significantly lower (that is, more blue). The mean values for the four groups include data from both the baseline and final evaluations. It does not indicate there was a significant change in H^* , only that the Chlortetracycline teeth were more blue than the other three groups.

Discussion

Bleaching treatment is considered to be the most conservative procedure for treating discoloured and stained teeth when compared to laminate and crown restorations. Moreover, bleaching can be used to reduce the colour of dark teeth before preparation and placement of aesthetic indirect restorations. Therefore, depending on colour reduction, the tooth preparation can be more conservative and preserve more sound dental tissues.^{7,12-14}

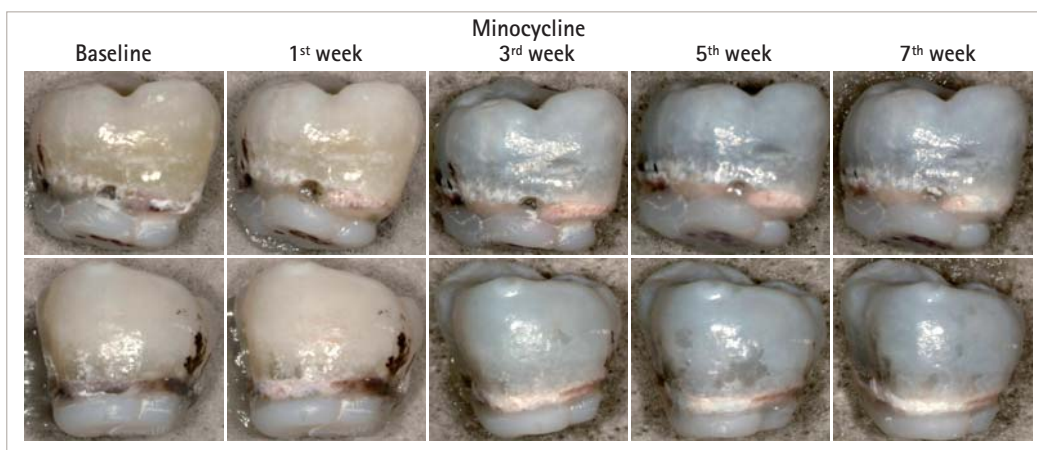


Fig. 3d

| | Baseline | | | 1 st week | | | 3 rd week | | | 5 th week | | | 7 th week | | |
|-------------------|----------|-------|--------|----------------------|-------|-------|----------------------|-------|-------|----------------------|-------|--------|----------------------|-------|--------|
| | L* | C* | H* | L* | C* | H* | L* | C* | H* | L* | C* | H* | L* | C* | H* |
| Chlortetracycline | 71.58 | 27.13 | 88.12 | 68.10 | 30.50 | 85.23 | 60.28 | 9.63 | 84.65 | 65.70 | 11.23 | 85.63 | 65.40 | 8.97 | 88.25 |
| Control | 81.73 | 20.17 | 100.03 | 82.07 | 23.02 | 94.48 | 73.20 | 11.97 | 97.77 | 87.25 | 16.98 | 101.33 | 85.52 | 15.33 | 99.60 |
| Doxycycline | 71.60 | 18.37 | 93.23 | 72.22 | 21.47 | 88.42 | 64.32 | 10.25 | 88.12 | 69.28 | 12.10 | 88.37 | 69.90 | 8.53 | 93.05 |
| Minocycline | 77.32 | 18.10 | 105.9 | 73.20 | 25.17 | 96.95 | 67.15 | 10.48 | 94.30 | 67.43 | 10.28 | 106.25 | 69.00 | 8.12 | 105.67 |

Table II

Table II_Means of colour difference at baseline and weeks 1, 3, 5 and 7.

Since its introduction to dentistry in 1989, night-guard vital bleaching has been proven to be a simple and safe procedure to lighten discoloured teeth. With extended treatment time, TCN-stained teeth can be expected to lighten in at least 86 % of cases. Side effects are usually mild and transient, disappearing within days of treatment completion with no long-term sequelae.¹⁵

Recent in-office tooth whitening procedures with 20 % hydrogen peroxide are gaining popularity as alternatives to at-home nightguard vital bleaching. The present study evaluated the efficacy of one such in-office whitening protocol on standardised *in vitro* stained human teeth.

One of the most significant observations is that visually the dark colour of the TCN-stained teeth appeared more intense as the treatment protocol progressed (Figs. 3a-d). The enamel appeared to be more translucent in all TCN groups, allowing the underlying darker dentine shade to be expressed. This is confirmed by the spectrophotometer readings in which the data shows a reduction in the L* values (Table II).

One explanation is that the superficial enamel layer was affected much faster by the higher concentration bleaching agent than the deeper dentine. Haywood hypothesised that dentine can be bleached in the case of Chlortetracycline-stained teeth.¹⁶ Success was achieved generally after two to six months of 10 % nightguard bleaching.^{17,18} In our case, the short treatment duration may not have resulted in sufficient contact time to effect dentine colour change. A slower process such as that suggested by Haywood may be indicated for teeth more intensely stained by tetracycline. Another explanation is that the colour of TCN-stained teeth became more intense in the artificial light owing to the photo-oxidation of this complex.¹⁹ However, there have been no studies on the efficacy or adverse effect of light in TCN-stained teeth.

The results support the conclusion that Zoom 2 provided a positive whitening effect, as one would

expect either a reduction in colour saturation and/or an increase in brightness. All four treatment groups demonstrated a reduction in C* from the baseline to final evaluations. Compared to the control group, the reduction in C* for the TCN groups was much more prominent. It is interesting to note that only the control group demonstrated an increase in brightness; all of the TCN groups decreased in brightness.

Conclusions

Subjecting the standardised TCN-stained teeth to the Zoom 2 in-office bleaching system yielded results consistent with a whitening effect when considering changes in chroma. However, a reduction in brightness was noted for all of the experimental groups, which is not consistent with the increase in brightness of the control group. Additionally, at the conclusion of the experimental period, the experimental groups attained a bluer hue when compared to their hue at the beginning of the experiment. No significant change in the hue of the control group was noted. Bleaching studies of teeth stained in the manner described in the present study may yield significantly different results to studies of teeth stained by TCN while they are developing.

Editorial note: A complete list of references is available from the publisher.

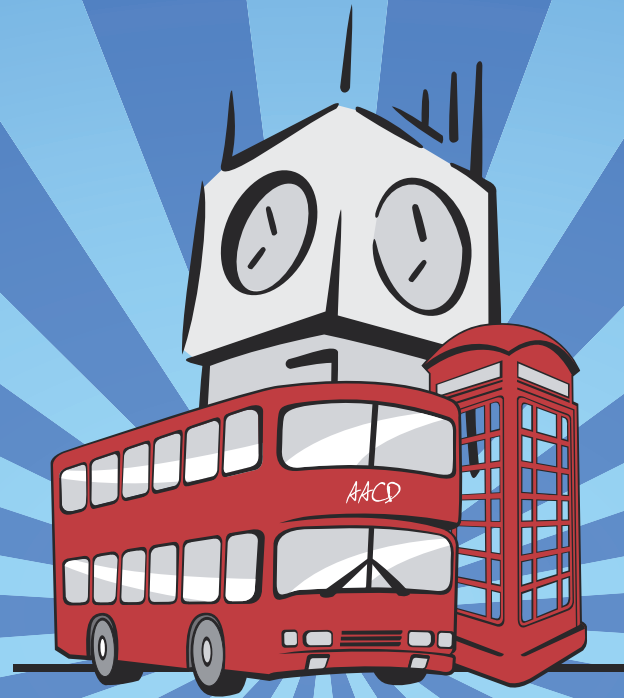
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