

Objective

Cariogenic biofilms and esterase originated from bacteria or saliva are relevant factors linked with failure of tooth-colored, resin composite fillings. The former, releases lactic acid that demineralize tooth structures, and the latter breakdown the ester bond present in polymerized methacrylate-based resin composites. Collectively, they create new cavities at the margins of the filling, i.e., secondary caries (SC). The aims of this study were to 1) determine the degree of conversion, and 2) *S. mutans* biofilm inhibition of an experimental acrylamide-based universal dental adhesive containing 2-aminoimidazole compound (2-AI-H10) and a commercial dental adhesive containing chlorhexidine (Peak Universal).

MATERIALS & METHODS

Experimental Design: the acrylamide-based universal dental adhesive system was comprised of HEAA, 10-MDP, BisGMA, UDMA, CQ, Ethanol and water. Monomers were added to separated amber flasks in order of most viscous to least viscous and stirred overnight. CQ was added followed by photo inhibitor, BHT, 4-methoxyphenol and stored into separated black plastic bottles at 4 °C. Such formulation was used to prepare the acrylamide-based universal containing no 2-AI/H10 (**control group**) and the experimental groups contain 2-AI-H10 at 2%wt and 6%wt.

Degree of Conversion (DOC): DOC was performed on unpolymerized (UN) and polymerized (P) materials using an ATR-FTIR instrument. For UN, 10µL were placed on a diamond ATR-crystal and absorbance spectra collected at 16 scans/1 cm⁻¹ resolution. For P, the same amount was placed on a glass slide, light cured (14 J/cm²) and spectra proceeded as with UN. The peak areas of methacrylamide and methacrylate C=C stretching at 1637 cm⁻¹ and aromatic reference peak area of Bis-GMA at 1608 cm⁻¹ of uncured and cured adhesives were used to calculate the DC% (n=5) using the adjacent formula.

Biofilm inhibition: *S. mutans* cell biofilm test was used to evaluate bacteria inhibition. Resin disks (N=3) of each material were prepared (1 mm high and 9 mm diameter), polymerized, and sterilized under UV light (8h) under a hood. Next, 1x10⁻⁵ bacterial *S. mutans* U159 were used with Tryptic Soy Broth for 24 h to grow early biofilm for 24h and 7 days. The disks were gently washed with PBS and a Live/Dead backlight kit was used to assess the biofilm viability using a Fluorescence microscope (Keyence Z1000, 20x). Live/Dead cell were counted using image cytometer and hybrid cell count.

RESULTS

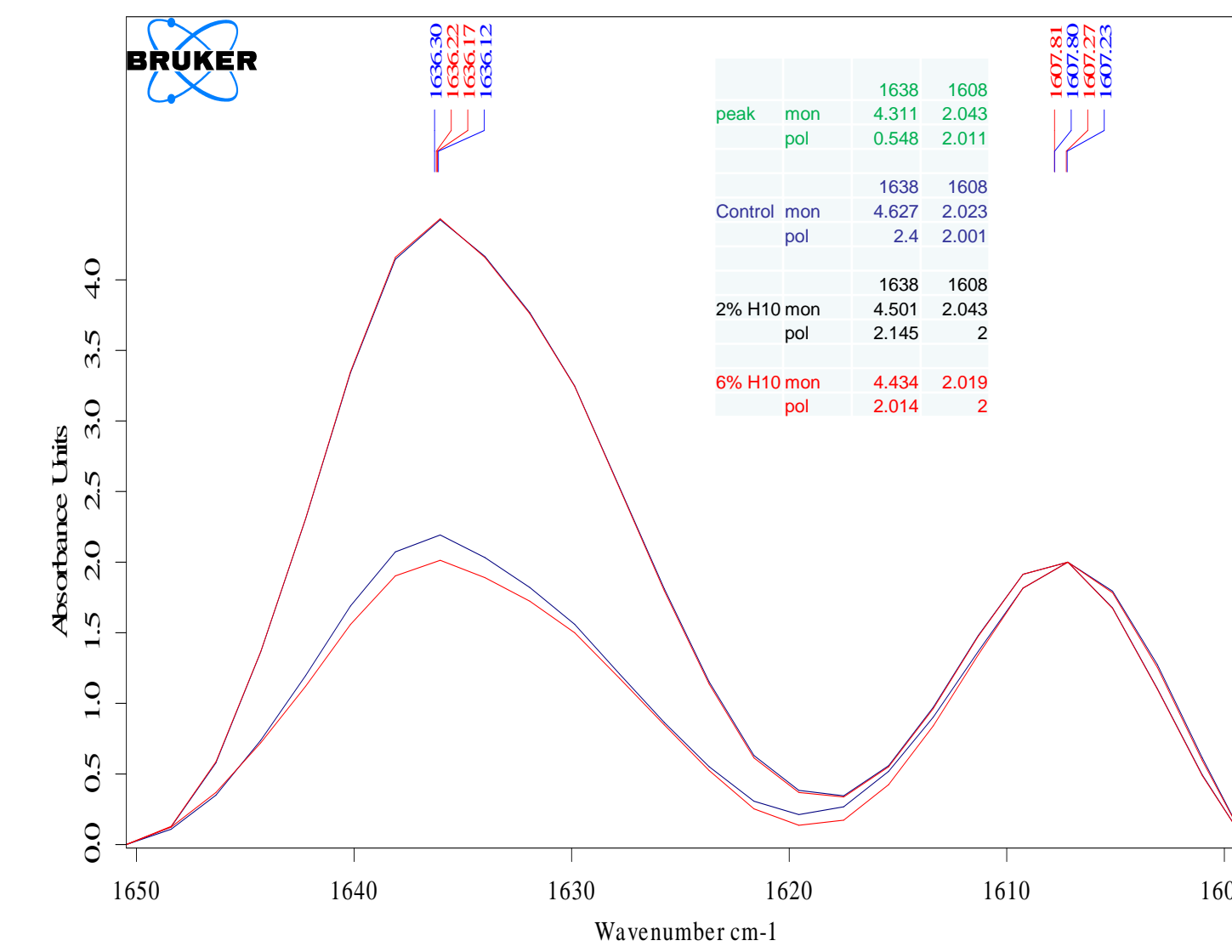


Figure 1. ATR-FTIR absorbance values for UP and P samples stretching at aliphatic (1637) and aromatic peaks (1608)

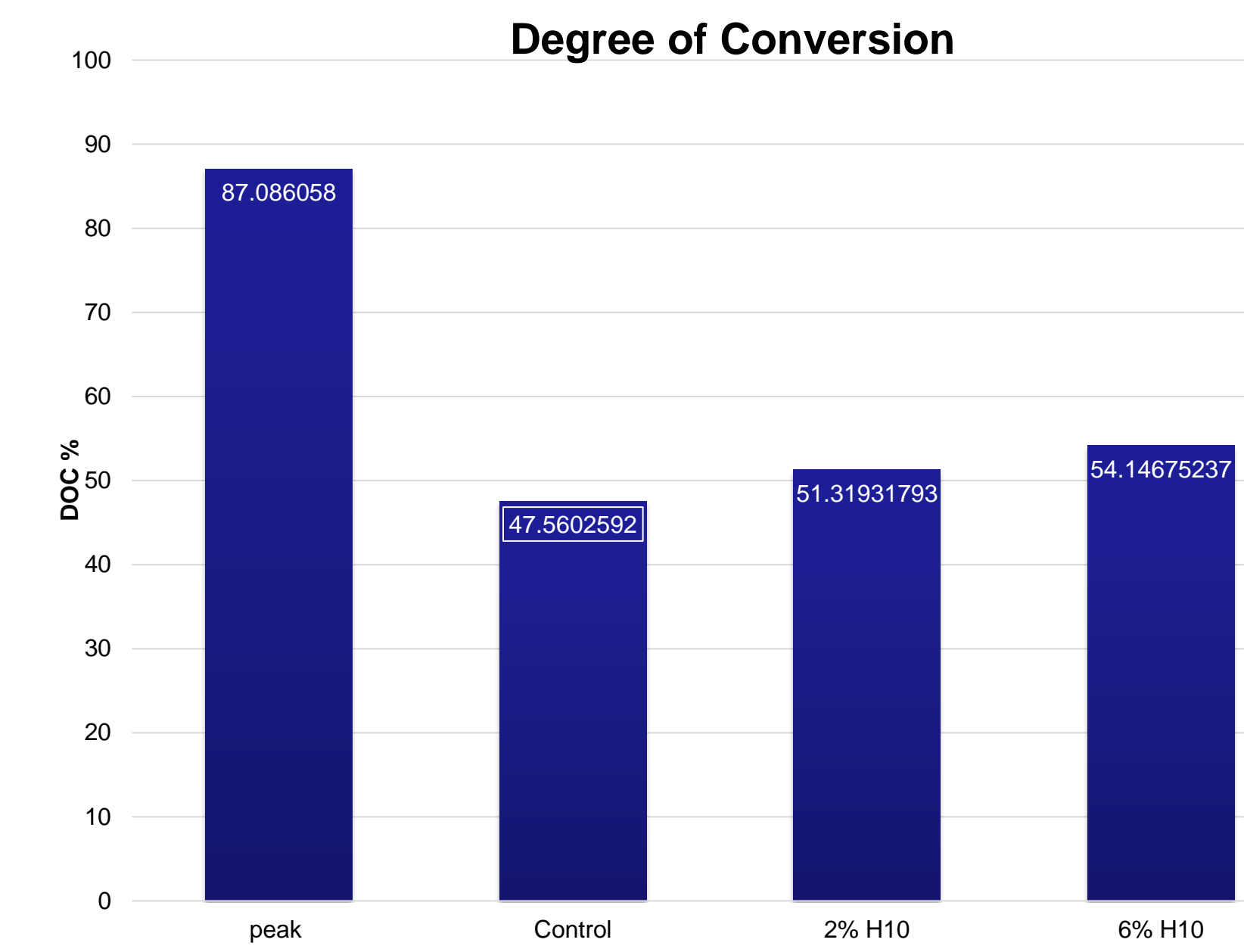


Figure 2. Degree of conversion results for all samples

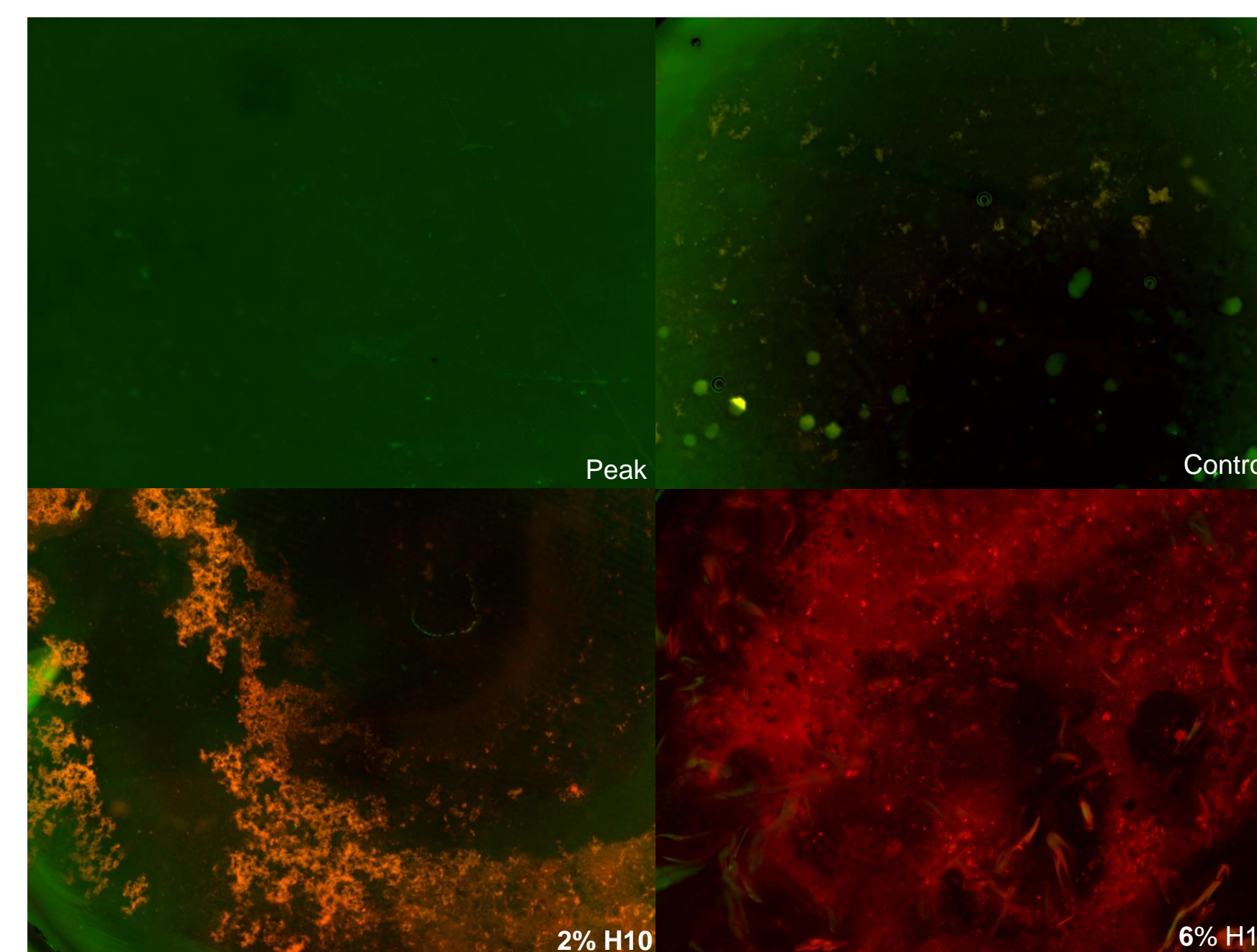


Figure 3. Fluorescence microscope biofilm production on P dental adhesive after 24 hours

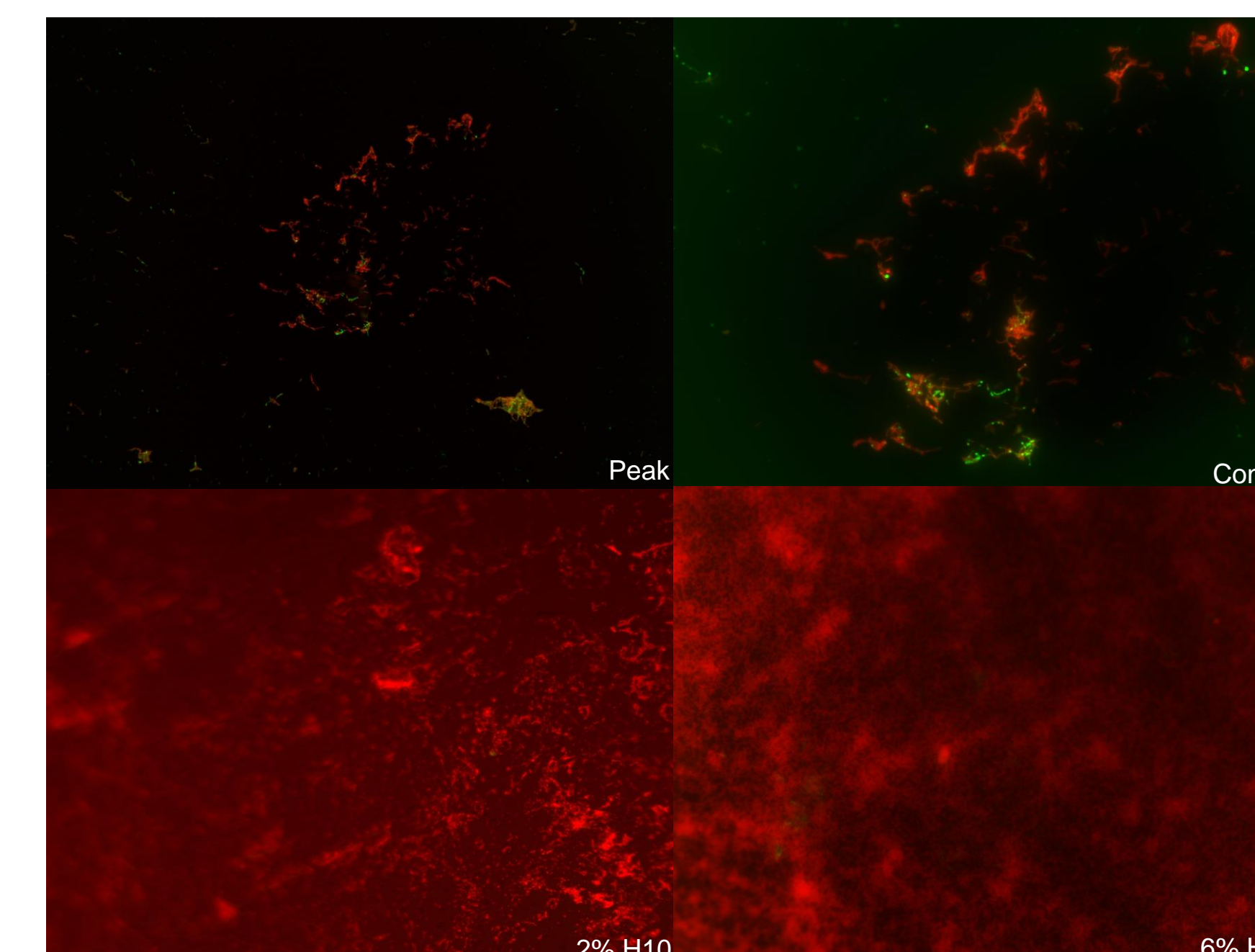


Figure 4. Fluorescence microscope biofilm production of P dental adhesive after 7 days

The degree of conversion were as follow: Peak SE (commercial) 87%, H10-acrylamide control 47%, H10-acrylamide 2% 51%, and H10-acrylamide 6% 54%. The *S. mutans* Live/Dead demonstrated that the universal adhesive containing 2-AI-H-10 compound, regardless of the concentration, diminished live cell count.

Conclusion and Further Study

It can be concluded that adding 2-AI/H-10 compound to acrylamide-based universal dental adhesive slightly increased the DC regardless of the concentration used, and that more live *S. mutans* cells were present in the control acrylamide-based material.

Much is to be determined about the use of 2-AI in dental adhesive. We are unsure if the presence of reproductively inactive bacteria of H10 containing samples correlate to decreased biofilm production. Further tests done to examine the microbiology of this interaction and current test should be replicated with a more stable solution to allow for bacterial statistical analysis. H10 should also be used in an acrylamide-based adhesive that resembles more of the commercial product. These samples containing the additional biomaterials for more bond strength and rigidity.

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DISCUSSION

The outcomes of the research have provided insight into the application of 2-AI/H10 in Acrylamide UA. In both 24 hours and 7 days, the antimicrobial inhibition of H10 has shown resilience in depleting functionality of bacteria as the concentration increases. The results for DC should be interpreted with caution to the limitations of the current research. The timeline did not allow for repetition of the test and therefore are negligent of the statistical significance.

REFERENCES

- Huigens, R. W., III, Richards, J. J., Parise, G., Balland, T. E., Zeng, W., Deora, R., & Melander, C. (2007). Inhibition of pseudomonas aeruginosa biofilm formation with bromoageliferin analogues. *J. AM. CHEM. SOC.*, 9(129). <https://doi.org/10.1021/ja069017t.s001>
- Nedeljkovic, I., Teughels, W., Munck, J. D., Meerbeek, B. V., & Landuyt, K. L. (2015). Is secondary caries with composites a material-based problem?. *Dental Materials*, 31(111). <https://doi.org/10.1016/j.dental.2015.09.001>
- Chen, L., Suh, B. I., & Yang, J. (2018). Antibacterial dental restorative materials: a review. *American Journal of dentistry*, 31(B), 6B-12B.
- Draughn, G. L., Allen, C. L., Routh, P. A., Stone, M. R., Kirker, K. R., Boegli L., Schuchman, R. M., Linder, K. E., Baynes, R. E., James G., Melander C., & Pollard, A., Cavanagh, J. (2017). Evaluation of 2-aminoinadizole variant as adjuvant treatment for dental bacterial infections. *Dove Press*, 11, 153-162.
- Ahmed, M. H., Yoshihara, K., Nagaoka, N., Yao, C., Matsukawa, A., Yoshida, Y., & Meerbeek, B. V. (2023). Acrylamide monomers in universal adhesives. *Dental Materials*, 39(3), 246-259. <https://doi.org/10.1016/j.dental.2023.01.003>