Dr. Dr. Kimberly Ngai - Antimicrobial Dental Material for Bacterial Inhibition and Caries Reduction

Dr. Kim Ngai: Hi, I'm Kim. I'm a third-year pediatric graduate student at the University of Toronto and today I'll be presenting my research project, which is on the Evaluation of Novel Antimicrobial-Containing Adhesive Systems for Bacterial Inhibition and Secondary Caries Reduction. As many of us know, resin composite is one of the most commonly used restorative materials in dentistry today. And this is due to increasing concerns regarding mercury exposure in dental amalgams, increasing demands for esthetic or tooth colored restorations, and of course it's versatile applications in day-to-day practice. However, despite this growing popularity in resin composite restorations, they do tend to lack the durability and have higher failure rates than dental amalgams and this can result in billions of dollars worldwide in replacement of restoration. And high failure rate is primarily due to the microleakage along the margins of restorations since as we know, an inadequate bond or seal between the tooth and the restorative material is susceptible to decay and can lead to secondary caries, as shown in this picture here. And so, there are many strategies that have been implemented to try and reduce the secondary caries process and improve the longevity of restorations. And in our focus, we are trying to incorporate antimicrobial properties within the adhesive resin, which is considered the weak link of any restoration.

Dr. Kim Ngai: And to quickly review the two types of resin adhesive systems, we have our total-etch and self-etch technique. Total-etch attempts to completely remove the smear layer, which is that accumulation of tooth debris at the bottom of our cavity preparations. And our second system is the self-etch system, where sometimes we get some etchant or priming agent remaining within the interface and can leak with a little bit of a smear plug within that dentinal tubule. Regardless of the total-etch system, our goal is to create a nice hybrid layer. It's that resin reinforced layer that helps with the bond between the composite and the dentin in our restorations. And so, our goal was to try to incorporate antimicrobial properties into each of these systems, so both total-etch and self-etch since both are used in clinical practice. And we developed drug-silica-particles where we assembled silica with an antimicrobial agent, which we used [inaudible] chloride in order to create these particles that we incorporated into our adhesive.

Dr. Kim Ngai: And we found with our DSP particles, we were able to overcome current challenges that antimicrobial systems right now have and that is kind of a short-term effect or burst release of the antimicrobial property as well as compromising mechanical structure after the release of the antimicrobial drug. And so with our DSPs, we showed in an in vitro study that there was long-term release and this was calculated to be well over 30 years as well as

biodegradation triggered release, meaning when bacteria attack was present, there was more release of the drug so that there was a greater effect when we actually needed it. And there was a significant reduction in the log bacterial counts at zero days and 30 days. And so, this was very promising, but the clinical efficacy of the DSP-loaded adhesives has yet to be examined and this is where our project comes along. So, our objective was to examine the efficacy of commercially modified total-etch and self-etch dental adhesives containing these drug-silica-particles in inhibiting interfacial bacteria biodegradation markers, which I'll go into a little bit more detail in a couple of slides and reducing the development of secondary caries.

Dr. Kim Ngai: How do we do this? We ran two separate experiments. The first one looked at the bacterial side of things. We wanted to look at the quality of the interface as well as the antimicrobial effectiveness of our drug. And our second experiment looked at secondary caries formation. So, we looked at demineralization and cavitation. Both experiments used the same specimen. So, we had commercial resin bonded to dentin from extracted molars and we used different adhesives to create four groups. So, we had two experimental groups, we have our DSP-loaded total-etch and self-etch. And two control groups, which is a stock total-etch and self-etch release.

Dr. Kim Ngai: For our first experiment, we incubated our specimen in simulated human salivary esterase or HSHE. And this was to induce biodegradation over 0, 90 and 180 days. So, we wanted to show the short- and long-term effect of our antimicrobial drug. After incubation we then transferred the specimen into a system called the chemostat valve implementer. And essentially it allowed us to have a lot of control over the conditions so we can control pH and temperatures so we could try to mimic the intraoral pathogenic environment and it allowed for constant inflow of media conditions. And we were able to create a dual species biofilm using streptococcus mutans as well as lactobacillus rhamnosus, which are two commonly found bacteria species in the oral cavity that have been associated with caries. So over three days we formed a biofilm which we then analyzed under confocal laser scanning microscopy.

Dr. Kim Ngai: So, this is an example of an image that you can, that we would see. And so the resin's at the top. Our dentin's at the bottom, here. And this is our resin-dentin interface. And after staining we were able to use quantitative analysis in order to get biomass, which is that total number of cells on the specimen surface. We we're able to calculate the live/dead ratio, which is the ratio between the viable which was stained green, and non-viable, which is stained red cells. And we we're also able to find out the depth of penetration. So how far from the surface of our specimen into the interface we're able to detect bacteria.

Dr. Kim Ngai: Our second experiment used the same [inaudible] as well as groups. And instead of trying to mimic the intraoral condition, we actually used an

accelerated caries batch model and so we were trying to induce caries in a relatively quick fashion. At about zero days we [inaudible] find micro CT analysis measures. And then we underwent our experiment where we incubated our specimen in media that was supplemented with sucrose and glucose as well as the same bacteria species from our first experiment. And what was different about this experiment was that at every 48 hours we replaced the media and we used fresh bacteria so that there was a constantly or almost constantly acidic environment that favored demineralization. And so, this created actually a greater cariogenic challenge than the oral cavity. And our reasoning behind this was that if our adhesive could withstand these harsh conditions, then when we actually used it in the mouth, we were hoping for similar or even greater effects.

Dr. Kim Ngai: And so, after 7 days of incubation we analyzed our specimens under micro CT and we used the axial view of our 3-D rendering in order to calculate our volumes of cavitation and demineralization. And what did we find? From our first experiment we ran a two-way ANOVA where we use drug and time as our independent variables, and we wanted to see if there was any interaction between the drug and time. And we found, and these are results for total-etch, that with our DSP-loaded adhesives there was a reduction in all bacteria by degradation markers. So, for biomass, again, which is that total number of cells that's on the tooth surface, there was a reduction. For live/ dead ratio there was a lower number, which means there is more non-viable cells. And this speaks to kind of the antimicrobial effectiveness of our drug.

Dr. Kim Ngai: And since the effect was similar at all time points, you know that there's long-term of bioavailability. And lastly, we looked at depth, the bacterial penetration. And just cause it's easier to visualize with a picture. For example, if we take, we're taking a look from the left side here, we're following the red arrows. So, we're trying to see how far into that interface we're able to detect bacteria and therefore calculate kind of the depth of bacterial penetration as well as how big that marginal gap was. And we found that with DSP-loaded adhesives, there was a reduction. And so, we found similar results in our self-etch group as well. The main difference was that at six months there's only significant reduction in depth of bacterial penetration, but all the other biomarkers were the same in terms of reduction. And so, you might now question what these reductions in biomarkers mean clinically.

Dr. Kim Ngai: And so that is why we ran our second experiment. And of course, it's not a one-to-one correlation in terms of the results because it is a different experiment with different specimens. But the results do compliment each other. And so, for our second experiment we found that there was a reduction in cavitation and demineralization. And they were all significant except for the demineralization volume in self-etch where it was trending significance with a P value of 0.052. And what was interesting to see was that for the cavitation volumes, the

reduction in terms of percentage was actually almost double that of demineralization. And it kind of gives you an idea of the role that DSP-loaded adhesives have in maintaining the integrity of the interface. And so, in conclusion, with the addition of drug-silica-particles to both the total-etch and self-etch adhesives, we found a reduction in interfacial bacterial biodegradation markers.

Dr. Kim Ngai: Again, this is for biomass viability and penetration. We saw a reduction in cavitation and demineralization, which is that clinical outcome that we're interested in. Because this is what's going to guide us in terms of if we need to replace a restoration or not. And we found that there was preservation of interfacial integrity as well as long-term bioavailability of the antimicrobial drug, which supports our previous findings. And so, our next steps would be to test our etches models total-etch and self-etch systems as well as in in vivo studies. And we currently have an animal in vivo study in progress where we're looking at the clinical effectiveness as well as the safety of these DSP loaded adhesives in rats. And hopefully we can perform future kind of clinical trials in humans.

Dr. Kim Ngai: I'd like to just quickly thank my supervisor, Dr. Yoav Finer, as well as my committee and lab members for helping me with this project. Of course, I couldn't do it without our collaborators and our grants. And I would just like to thank you guys for listening.