

# Development of in vitro Multispecies Biofilms with Hydroxyapatite and Artificial Saliva

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The purpose of this study was to develop an in vitro multispecies biofilm that had more resemblance to the human oral biofilm than the models that were previously in use. It was hypothesized that coating plates with hydroxyapatite and artificial saliva would increase the amount of early colonizers in biofilm and would decrease the number of monopolizing bacteria, making the model more realistic through changes in surface properties. A total of 42 species of bacteria were grown on 192 pegs set in broth. Of these pegs a fourth were coated in both hydroxyapatite and artificial saliva, a fourth were coated with hydroxyapatite, a fourth were coated with only artificial saliva, and a fourth were the control, with nothing coated on the plastic pegs. Samples were collected every day from day 4 to day 9. They were analyzed by four methods: Checkerboard, ROQT, scanning electron microscope imaging, and genome sequencing. The hypothesis was partially supported in that adding hydroxyapatite not only increased general bacterial amounts and variety but also decreased the level of monopolizing bacteria. However, early colonizers weren't reinforced and saliva only improved the biofilm near the beginning of the experiment. Nonetheless, hydroxyapatite coated pegs grew biofilms that were closer to human oral biofilms, so these could be utilized in future experiments. This improved model could be used to assess antimicrobial drugs, investigate mechanisms in biofilm formation, and study biofilm interactions with human cells.