

# ***Ultrastructural Study of Plaque Biofilms in an Invisalign® Orthodontic Appliance***

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Invisalign® appliances are sequential medical-grade, translucent, polycarbonate tooth positioners designed to produce orthodontic tooth movement. So far, no studies have been performed to investigate the nature of biofilms on these appliances. Objectives: An observational study on the ultrastructural features of plaque biofilms on fitting surfaces of a two-week old Invisalign® appliance worn by a patient. Methods: An adult patient undergoing Invisalign® therapy for 6 months, changing the aligners every two weeks. A two-week old upper appliance was collected and preserved. The sample was sectioned into 8 sections, and sputter coated with elemental gold. These were then examined using a conventional Scanning Electron Microscope using magnifications ranging from 30x to 4,000x. Results: Low magnifications revealed macro and microscopic corrugated surface morphology of appliance, consistent with its laser stereolithography modulation. At low magnifications biofilms at all stages of development were seen although some areas were totally devoid of organisms. Biofilms were particularly noticeable in sheltered areas and crevices. Bacterial species noted included coccal and bacillary forms, and filamentous species. At higher magnification, mature plaque samples revealed archetypal biofilm structure, comprising mixed, sessile, bacterial forms embedded in an extracellular polymeric material and arranged in characteristic "tower" formations as well as flat sheets. Large amounts of interlinking extracellular proteoglycan and numerous pores/channels for aqueous and ion/nutrient/messenger exchange were also noted. The colonial architecture of the biofilms varied from discrete, single species, "pioneer" colonies to fully mature plaque comprising multi species colonies coexisting in a single habitat. Conclusions: These preliminary studies reveal for the first time structural details of the Invisalign® appliances and ultrastructure of the biofilm architecture on such appliances.

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