



Biofilm growth on orthopedic implantable materials: static or dynamic condition what is the most appropriate methodological tools to study device-related infections?

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BACKGROUND

Surface-associated biofilms

Bacteria can form submerged biofilms under both static and shear-flow conditions. These types of biofilms are one of the most relevant in most chronic infectious disease states [1, 2]. It is estimated that up to 80% of microbial infections in the human body involve biofilm formation, greatly contributing to morbidity and mortality, especially in hospital settings [3,4]. Biofilms can also develop on abiotic surfaces, including medical devices such as orthopedic prostheses, artificial cardiac valves, coronary stents, intravascular and urinary catheters, neurosurgical, cochlear, and breast implants, dentures, and ocular devices [5].

Culturing Biofilms under Flow Conditions

Continuous-flow cultures enable the formation of mature biofilms in chambers covered with coverslips or on silicone or latex tubes fitted to a peristaltic or syringe pump. The peristaltic pump facilitates flow of fresh growth medium, whereas planktonic cells and waste are removed. These flow systems create optimal conditions for the generation of mature biofilms. Culture preparation, surface conditioning, and adjusted methods provide lab substrates mimicking clinical conditions. A characteristic example involves the evaluation of four CVC *Staphylococcus epidermidis* biofilm infection models that differ in material type (glass versus polymer) and nutrient presentation (static versus continuous flow) [6].

PURPOSE AND HYPOTHESIS

Purpose

Study of biofilm growth under static and dynamic conditions to evaluate the most suitable orthopedic materials on the prevention of device-related infections.

Hypothesis

- Null Hypothesis: no differences of biofilm growth for each different materials.
- Alternative Hypothesis: there are differences of biofilm growth for each different materials.

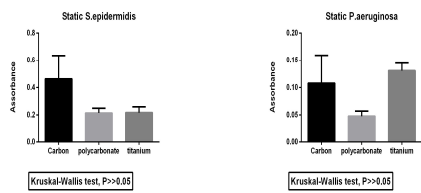
MATERIALS AND METHODS

Biofilms of *Staphylococcus epidermidis* (ATCC 35984) *icaA* and *icaD* genes positive and *Pseudomonas aeruginosa* (DSM 939) were generated under static and dynamic conditions, adding the bacterial inocula on titanium, carbon, polycarbonate, 316 stainless steel and carbon-peek coupons housed in flat bottom test tubes or in the Biofilm Reactor system respectively. Biofilm growth was evaluated by MTT assay after 48 hours.

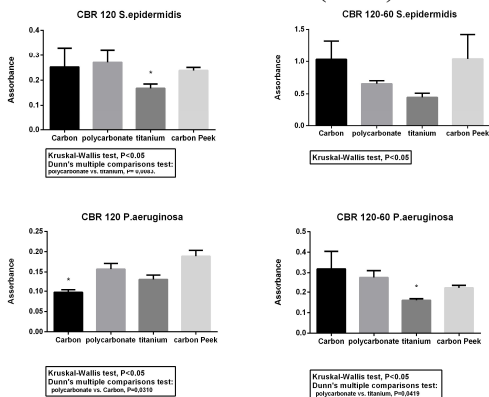
RESULTS

Results of dynamic model showed a better capacity of *S.epidermidis* to grow with a rotation between 120-60 rpm on each tested materials (Mann-Whitney test, p-value < 0,05) than *Paeruginosa*. Titanium was the material on which the bacterial strains adhered less, whereas carbon and polycarbonate allowed greatest adherence of *Paeruginosa* (Mann-Whitney test, p-value < 0,05). Results of static model showed that both species grew on each materials without distinction (Kruskal-Wallis test, p-value 0,95). *S.epidermidis* growth was better also under static condition.

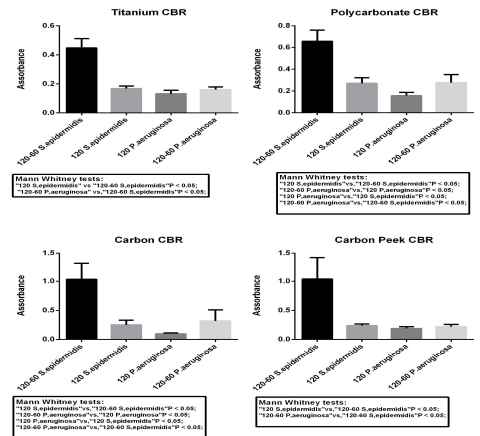
Static Model



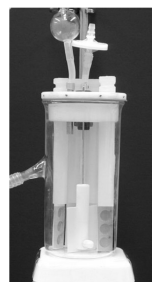
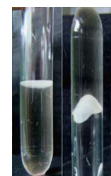
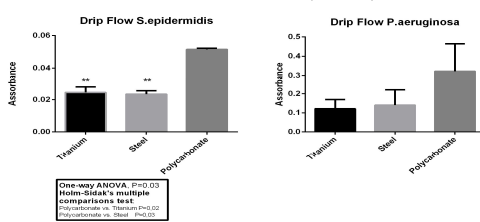
Dinamic Model (CBR)



Dinamic Model (CBR)



Dinamic Model (DFR)



CONCLUSIONS

The static model was not able to evaluate the different adhesion capacity of the strains to the materials, confirming the dynamic model is the most suitable tool for the study of orthopedic materials on the prevention of device-related infections.

BIBLIOGRAPHY

- 1-Thomsen T, Hall-Stoodley L, Moser C, Stoodley P. 2011. The role of bacterial biofilms in infections of catheters and shunts, p 91–109. In Bjarnsholt T, Jensen PO, Moser C, Hoiby N (ed), *Biofilm infections*. Springer, New York, NY.
- 2-Foxman B. 2014. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect Dis Clin North Am* 28:1–13.
- 3-J.W. Costerton, P.S. Stewart, E.P. Greenberg. Bacterial biofilms: a common cause of persistent infections, *Science* 284 (1999) 1318–1322.
- 4-I. Francolini, G. Donelli, Prevention and control of biofilm-based medical-device-related infections, *FEMS Immunol. Med. Microbiol.* 59 (2010) 227–238.
- 5-Perival SL, Suleman L, Vuotto C, Donelli G. 2015. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. *J Med Microbiol* 64:323–334.
- 6-Van Kerckhoven M, Hottelbeekx A, Lanckaerck E, Moons P, Lammens C, Kerstens M, Ieven M, Delputte P, Jorens PG, Malhotra-Kumar S, Gossens H, Maes L, Cox P. 2016. Characterizing the in vitro biofilm phenotype of *Staphylococcus epidermidis* isolates from central venous catheters. *J Microbiol Methods* 127:95–101.

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