－ Original Article －

An *in vitro* model for a packed bacterial mass formation with special attention to less interstitial spaces

Hiba A. Al-Shawafi[1)] , Hiroyuki Uematsu[1)] , Shoji Takenaka[2)] , Etsuro Hoshino[1)]

[1)] *Division of Oral Ecology in Health and Infection, Niigata University Graduate School of Medical and Dental Sciences (Chief: Prof. Etsuro Hoshino)*
[2)] *Division of Cariology, Operative dentistry and Endodontics, Niigata University Graduate School of Medical and Dental Sciences*

細菌間間隙の少ない，細菌密度の高い細胞集団の作成モデル

Hiba A. Al-Shawafi[1)] , 上松弘幸[1)] , 竹中彰治[2)] , 星野悦郎[1)]

[1)] 新潟大学大学院医歯学総合研究科　口腔環境・感染防御学分野（主任：星野悦郎教授）
[2)] 新潟大学大学院医歯学総合研究科う蝕学分野

平成 23 年 3 月 23 日受付　　5 月 27 日受理

Key words：bacterial mass, biofilm, *Enterococcus faecalis*, interstitial spaces, *Propionibacterium acnes*

Abstract：

Objective: This study aimed to establish a new model for bacterial mass formation with special attention to less interstitial spaces, in other words, a model for a well-packed bacterial mass.

Materials and methods: *Enterococcus faecalis* or *Propionibacterium acnes* microorganisms were centrifuged onto a cover glass, and allowed to stand for 60 min under 95% humidity. Then, the bacteria were cultured under restricted supply of media (25 μl at 60 min intervals) for 72 h for *E. faecalis* aerobically and for 5 d for *P. acnes* anaerobically. Excess media were immediately removed using sterilized absorbing papers, so the media stayed in the layer where bacteria were growing.

Results: After the 60 min-standing, the centrifuged bacteria were firmly adhered on the glass surface, whereas those without 60 min-standing were washed away. The bacterial density in the cultured bacterial mass was higher than in those formed by centrifugation or filtration, meaning that bacteria might multiply to throng and fill up the inter-bacterial spaces to make a packed monolayer, then, piled up to make the second layer, and continued one after another. When excess culture media were supplied, bacteria formed a less packed bacterial mass.

Conclusion: The well-packed bacterial mass seemed to be quite equivalent to the so called "biofilm" in regard to insufficient infiltration of molecules into the bacterial mass, although the constituting bacteria do not produce extracellular polymers.

抄録：

本研究は細菌間の間隙の少なさに注目した細菌塊形成の新しいモデル，言い換えれば，細菌同士が密着した細菌塊の形成モデル，を確立することを目的としている。*Enterococcus faecalis* あるいは *Propionibacterium acnes* の生菌をカバーグラス上に遠心によって付着させ，湿気を保って 60 分放置後，*E. faecalis* の場合 72 時間にわたって好気的に，*P. acnes* の場合 5 日間嫌気的に，1 時間毎に 25 μl の培地を供給した。余分な培地は直ぐに滅菌した吸収紙を用いて除去し，遠心付着させた 1 層の細菌層の高さに培地が供給され，その中で細菌が増殖するようにした。60 分間の放置中に細菌はカバーグラスに強く付着したと思われ，放置しなかった場合，容易に剥がれて洗い流された。この様にして形成された細菌塊は，遠心細菌塊や濾過細菌塊に比べ細菌密度が高く，細菌が密接して細菌間のスペースが無い状態で増殖し，その後，その上にまた密な細菌層を形成していると思われた。この様に密に形成された細菌塊は，細胞外に多糖体を形成しないにもかかわらず，いわゆる「バイフィルム」と同様，物質の浸透が極めて悪い状態にあると思われた。