

Adsorption Effect of Hydroxyapatite to Oral Streptococci

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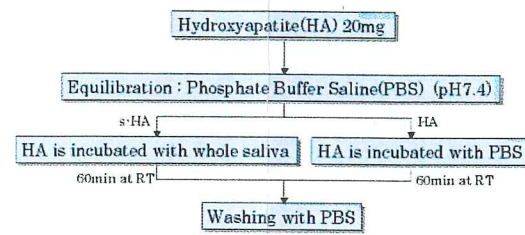
Aim of Study

Hydroxyapatite(HA) is a component of biological hard tissue, including tooth enamel. It has high ability to adsorb oral streptococci. To investigate whether HA might be a useful agent for a Dental Drug Delivery System (3DS), a new system for specifically removing oral streptococci from the oral cavity, the relationship between bacterial adsorption and the physical form of HA prepared by heating at different temperatures (0, 200, 800 and 1200 C) was analyzed by in vitro assay using 550nm light absorbance.

Materials and Methods(1)

[Adsorption assay]
Each of the four different forms of HA was added to a PBS solution of oral streptococci (Streptococcus mutans, S. sobrinus, S. sanguis, S. mitis, S. salivarius, and S. anginosus respectively) and incubated for 90 min at 37 C. The procedure was then repeated using the same forms of HA coated with saliva (s-HA). After standing of the mixtures at room temperature, the supernatants were analyzed for differences in optical density before and after addition of HA or s-HA, to assess bacterial adsorption levels. The effect of adding divalent ions to the PBS on S. mutans adsorption to HA or s-HA was also investigated. Bacterial adsorption to HA was also observed by SEM.

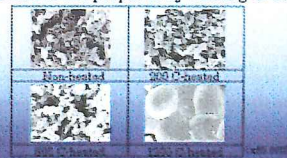
Treatment of HA



Materials and Methods(2)

[Bacteria]
Streptococcus mutans MT8148
// sobrinus 6715
// sanguis ATCC10556
// mitis ATCC6249
// salivarius ATCC9759
// anginosus ATCC33397

[Different forms of HA prepared by heating at different temperatures]



Assay for adsorption to s-HA and HA

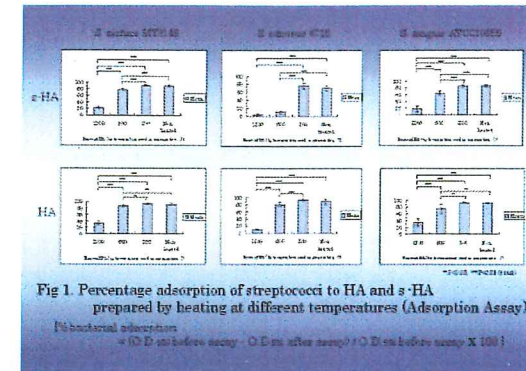
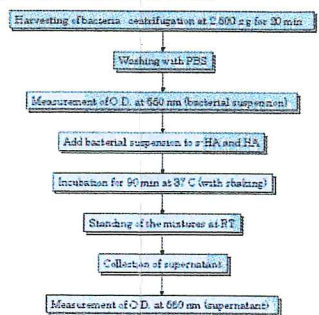


Fig 1. Percentage adsorption of streptococci to HA and s-HA prepared by heating at different temperatures (Adsorption Assay)

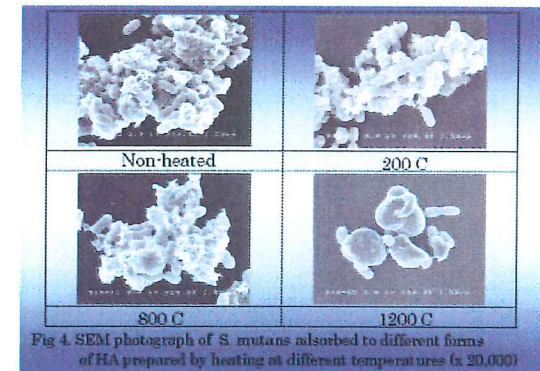


Fig 4. SEM photograph of S. mutans adsorbed to different forms of HA prepared by heating at different temperatures (x 20,000)

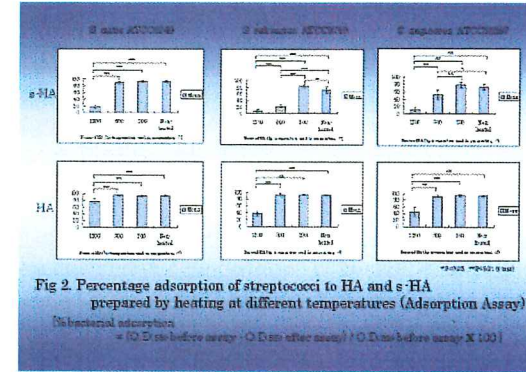


Fig 2. Percentage adsorption of streptococci to HA and s-HA prepared by heating at different temperatures (Adsorption Assay)

Results

1. Adsorption of bacteria to HA was highest for forms of HA prepared using low or no heat-treatment. Non- and 200 C-heated HA tended to show the strongest adsorption to all streptococci.
2. Adsorption of bacteria to s-HA differed according to type of bacteria. Non- and 200 C-heated s-HA showed relatively high adsorption to S. mutans and S. mitis, but relatively low adsorption to S. sobrinus and S. salivarius.
3. In tests for the effect of adding divalent ions to the adsorption assay solution, Ca²⁺ lowered the adsorption of S. mutans to three of the four forms of s-HA, but Mg²⁺ had no significant effect. In contrast, both Ca²⁺ and Mg²⁺ raised the adsorption of S. mutans to HA, but only in the case of 1200 C-heated HA.
4. SEM observation suggested that bacterial adsorption levels may be dependent on the crystal size of different forms of HA.

3DS(Dental Drug Delivery System)



Model of bacteria removal by the combined use of P3ATC and 3DS
 (1) Even after biofilm is physically removed by P3ATC, bacteria remain on tooth surface
 (2) Drug-retainer containing antibacterial agent is used to eliminate remaining bacteria
 (3) Bacteria are completely removed

Function of drug-retainer
 Only a small amount of antibacterial agent is required to penetrate into all corners
 (1) Antibacterial agent is placed in drug-retainer
 (2) Drug-retainer with antibacterial agent is fitted over tooth surface

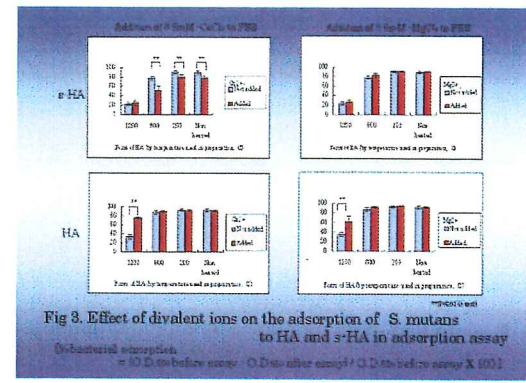


Fig 3. Effect of divalent ions on the adsorption of S. mutans to HA and s-HA in adsorption assay

Conclusions

1. HA prepared under low or no heat treatment shows the greatest potential for adsorbing streptococci in the oral cavity.
2. Saliva-coated HA (s-HA) shows lower adsorption to streptococci than saliva-free HA. This supports potential use of HA as a bacteria-removing agent in 3DS, where it can be administered in a drug-retainer that keeps the agent saliva-free.
3. Use in a drug-retainer closely fitted to the teeth also suggests HA could be employed to selectively remove oral streptococci, without affecting other oral bacterial flora.
4. Our results suggest strong potential for using non- or low heat-treated forms of HA as bacteria-removing agent in 3DS.