ANTIBACTERIAL ACTIVITY OF FLUORIDE COATED HYDROXYAPATITE GRAFT - AN ADDED ADVANTAGE

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ABSTRACT

BACKGROUND
As per WHO, a rough estimate 10–15% of worldwide population suffer from the periodontal disease, which is caused by roughly more than 500 microbes, most of them are commensals. The regenerative treatment for alveolar bones has increased many folds, but still have limitations.

MATERIALS AND METHODS
Fluoride incorporated Hydroxyapatite has been formulated and antimicrobial property is checked against control strains of S. mutans and S. oralis with double dilution method.

RESULTS
The Fluoride incorporated Hydroxyapatite has an excellent antibacterial activity and is able to reduce the number of bacterial count, both at pH 5 and pH 7.

CONCLUSION
Fluoride with bone graft can have added advantage over many alloplast. Its antibacterial property shown in present study can be a building block for further evaluation of its property on periodontal pathogen.

KEYWORDS
Periodontal infection, Alveolar bone loss, Fluoride incorporated Hydroxyapatite.

MATERIALS AND METHODS

The present in vitro study was approved by the Institutional Ethical Review Board, JSS Dental College and Hospital, an affiliated institution of the JSS University, Mysore. This study was conducted at Department of Pharmaceutics, JSS College of Pharmacy and Department of Microbiology, JSS Medical College and Hospital, an affiliated institution of the JSS University, Mysore.

Formulation of Fluoride Incorporated Hydroxyapatite

FA-coated HA graft was formulated according to methodology given by Nordquist WD et al \(^6\) at JSS College of Pharmacy, Mysore. Resorbable Hydroxyapatite graft (HA) (300-500 μm) was obtained from Eucare Pharmaceuticals (Sybograf). Sodium fluoride salt was obtained for the formulation of fluorapatite-coated HA graft. Procedure for fluoride incorporated Hydroxyapatite graft formulation:

- 4% sodium fluoride (NaF) solution was prepared.
- Hydroxyapatite graft mixed and reacted for 2 mins.
- The excess fluoride solution was drawn off.
- The FA-coated HA powder washed 3 times with distilled water.
- The powder was then oven dried at 80°C-100°C.

Gamma sterilisation and packaging was done at Eucare Pharmaceuticals, Chennai.

Confirmation of Fluoride Incorporation in Hydroxyapatite Graft Energy Dispersive X-Ray (EDX)

Analysis with spectroscopy was done for the confirmation of FA-coating on HA graft. Fluoride releasing pattern assessment in normal saline was done at Sri Jayachamarajendra College of Engineering, Mysore in Department of Environmental Sciences; 0.5 g of Fluorapatite coated Hydroxyapatite bone graft was mixed in distilled water and assessed at 24 and 48 hours with Sension 4 pH/ISE/mV laboratory pH/ISE/MV Meter.

Microbiological Analysis

The microbiological study was carried out in the Department of Microbiology, JSS Medical College and Hospital. Microbial strains \(S.\) mutans (ATCC25175) for Tooth associated infection and \(S.\) oralis (ATCC9811) for tissue associated infection were procured. Standard microbiological procedures were carried to make the double dilution of the Fluorapatite coated Graft solution. The graft was left overnight in 7pH solution and the serial dilution of solution was prepared for the procedure. Cultured microbial strains were incorporated and inoculated for 24 hours. Similar procedure was done at 5pH also in 1:1 dilution. A controlled tube was kept with normal saline in both the procedures.

RESULTS

Energy Dispersion X-Ray Analysis

Confirmation of coating of Fluoride in Fluorapatite coated Hydroxyapatite was done using Energy Dispersion X-Ray Spectroscopy and it was observed that distinct fluoride peak was seen at 0.70 KeV, which was not found in Hydroxyapatite graft (Fig. 1-4). By weight percentage amount of Fluoride was 0.66 in Fluoride coated HA (0.85 by amount percentage) against nil in Hydroxyapatite.

Fluoride Release Pattern

The in vitro fluoride releasing pattern in present study was evaluated. The concentration of Fluoride released from Fluorapatite coated Hydroxyapatite prepared bone graft in Distilled Water increased gradually from 1.67 ppm at 24 hours to 2.07 ppm at 48 hours.

Antimicrobial Analysis

At pH 7, turbidity or growth was seen in \(S.\) mutans strain at all the dilutions like 1:1, 1:2, 1:4, 1:8 including the controls. In case of \(S.\) oralis the tubes having a dilution of 1:1, 1:2 had no turbidity or growth observed while tubes with dilution 1:4 and 1:8 including controls were having turbidity/growth. (The results obtained are as mentioned as in Table 1).

At pH 5 there were no growth or turbidity observed by \(S.\) mutans or \(S.\) oralis, while the control tubes were observed having a turbidity at 1:1 dilution. (The results obtained are as mentioned as in Table 2).

**Fig. 1: Energy Dispersion X-Ray Analysis of Hydroxyapatite Crystalline Graft (Without any Fluoride Peak). Energy Dispersion X-Ray Analysis of Fluorapatite Coated Hydroxyapatite Crystalline Graft (With Small Fluoride Peak because of Fluoride**

**Fig. 2: Energy Dispersion X-Ray Analysis of Fluorapatite Coated Hydroxyapatite Crystalline Graft (With Small Fluoride Peak because of Fluoride Molecular Weight)**
The results of the present study could not be compared with past studies due to its distinctive approach and design. But HA is being extensively used in periodontal surgery as well as fluoride for its antibacterial property for numerous bacteria. Hydroxyapatite alloplast has been proven to be useful for augmentation procedures in osseous defects in the peri-implantitis lesions and intraosseous periodontal defects. And Fluoride exhibits antibacterial activity against a wide range of putative periodontal pathogens Streptococcus species, Treponema denticola, Lactobacillus, Actinomyces species. A study has been done to determine the minimum inhibitory concentration of Fluoride solution for 45 bacterial strains, out of which 22 were oral species and found that some bacterial strains like Actinobacillus actinomycetemcomitans and Capnocytophaga had susceptibility at very low concentration of Fluoride solution. So, fluoride incorporating in the existing bone graft substitute could be beneficial in restricting bone graft contamination in the infected periodontal site.

An interesting finding has been discovered about Fluoride is that, it not only have antibacterial activity but cell proliferation activity as well. The release pattern of Fluoride from formulated HA graft was little higher (1.67 and 2.07 ppm at 24 and 48 hours respectively) than that of the similar study done in Yokosuka, Japan. But the amount of fluoride released was within the range of its ability to have osteoblastic cell proliferation activity, as it was studied previously. The antibacterial property and osteoblastic cell proliferative activity of Fluoride can be provided a synergistic effect to HA, which is one of the most commonly used graft material.
CONCLUSION
Within the limits of present study, it can be concluded that Fluoride with bone graft can have added advantage over many alloplast. Its antibacterial property shown in present study can be a building block for further evaluation of its property on periodontal pathogen.

REFERENCES