Antimicrobial Properties of 45S5 Bioactive Glass Particulate

David M. Gaisser, David C. Greenspan*

NovaBone Products, LLC, 13709 Progress Blvd., #33, Alachua FL 32615

** Novamin Technology, Inc., 13709 Progress Blvd., #23, Alachua, FL 32615*

Introduction

Reports collected from clinical use over the last ten years have indicated that 45S5 Bioglass® bone grafting devices may have intrinsic antimicrobial properties, as evidenced by anecdotal reductions in infection rates when used in oral osseous defects. To evaluate the reported response, a standard *in vitro* analysis for antimicrobial effectiveness was conducted on a Bioglass particulate device. The results of this testing are reported.

Materials and Methods

PerioGlas (NovaBone Products, LLC), a bioactive glass device with a particle size range of 90-710 µm, was evaluated using the USP <51> Antimicrobial Effectiveness Test. Cultures of five common organisms were exposed to PerioGlas particulate and evaluated for the resulting number of colony forming units (cfu) at periods of 7, 14, and 28 days. The organisms evaluated were:

- E. coli (ATCC 8739)
- P. aeruginosa (ATCC 9027)
- S. aureus (ATCC 6538)
- C. albicans (ATCC 10231)
- A. niger (ATCC 16404)

Changes in organism concentration documented the effectiveness of Bioglass to act as an antimicrobial agent.

Results

Tables I and II summarize the results of the current test. The results in Table I present the microbial concentration at test start and each subsequent time period after exposure to Bioglass particulate. Table II reports the relative change in microbial count, shown as the log reduction from original concentration.

Results (cont.)

Table I. Microbial Concentration of Bioactive Glass Cultures as a Function of Time (CFU/ml product)

Time	E. coli	P. aer.	S. aur.	C alb.	A. nig.
Start	$1.4x10^6$	$3.9x10^6$	$1.2x10^6$	$2.8x10^4$	$8.1x10^4$
7 days	$7.6x10^2$	$1.0x10^5$	$1.0x10^1$	$1.8x10^2$	$2.6x10^4$
14 days	$5.0x10^1$	$<1.0x10^1$	$2.9x10^3$	$<1.0x10^1$	$2.5x10^4$
28 days	$<1.0x10^1$	$1.1x10^4$	$2.3x10^4$	$2.0x10^1$	$5.7x10^2$

Table II. Log Reduction in Microbe Content on Exposure to Bioactive Glass Cultures as a Function of Time

Time (days)	E. coli	P. aer.	S. aur.	C alb.	A. nig.
7	3.2	1.6	5.1	2.1	0.5
14	4.4	>5.6	2.6	>3.4	0.5
28	>5.1	2.6	1.7	3.1	2.1

At the earliest time point of 7 days, bioactive glass particulate was effective in achieving an average 2.5-log reduction in concentration for all microbes tested. This ranged from a 0.5 log reduction for the fungus A. niger to an almost complete reduction in S. aureus (to $1.0x10^1$ cfu/ml). Additional decreases in microbial count were seen at 14 days for the E. coli and P. aeruginosa bacteria and for the yeast C. albicans, with the C. albicans and P. aeruginosa decreasing to the $1x10^1$ level previously observed for S. aureus. By 14 days, however, the S. aureus level had begun to increase. By 28 days, all microbe levels were

Results (cont)

lower than the initial concentrations, with an average 2.9 log reduction. However, while the microbe content continued to decrease for the E. coli and A. niger, the levels for the remaining microbes had increased over their 14-day values. This is graphically represented in Figures 1-5; the bars charts represent the microbial count, while line charts denote the percent reduction in microbes from time zero.

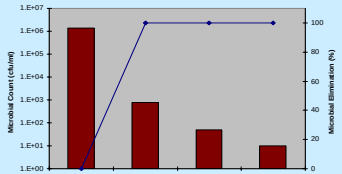

Figure 1. Microbial count with time in culture – E. coli

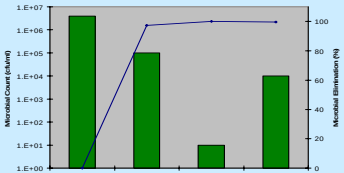

Figure 2. Microbial count with time in culture – P. aeruginosa

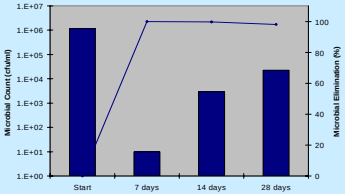

Figure 3. Microbial count with time in culture – S. aureus

Results (cont)

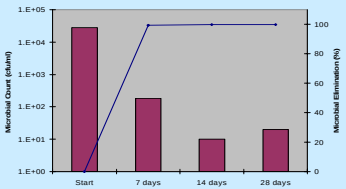

Figure 4. Microbial count with time in culture – C. albicans

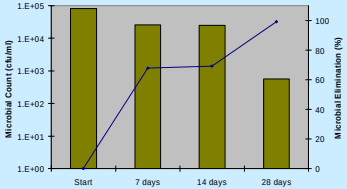

Figure 5. Microbial count with time in culture – A. niger

Discussion

The USP Antimicrobial Effectiveness test was developed for evaluating materials to be used as a long-term preservative. It is not specifically designed to evaluate whether a material has an antibacterial function. To meet the test requirements to be defined as an antimicrobial preservative, a material must demonstrate a specific reduction in all microbes evaluated and maintain this reduction for a period of 28 days with no increases at later time periods. While 45S5 Bioglass did not meet this requirement, it was demonstrated that particulate bioactive glass was effective in obtaining an initial reduction in the levels of all the microbes tested. Without complete elimination of the microbes, however,

Discussion (cont)

the microbial level subsequently were observed to increase at the latter time periods. This would indicate that the antimicrobial properties of the bioactive glass particulate are transitory.

The results of this testing are in agreement with prior short-term tests on bioactive glass particulate. Both Allan et al.(1), and Stoor, et al.(2), tested bioactive glass against common oral organisms. These tests indicated a strong anti-microbial function, but they were only conducted for periods of up to 3 hours. It was theorized that this effect was due to local osmotic effects, pH changes and/or a rise in calcium concentration, due to the release of ions from the particulate material surfaces. The current test supports these findings, and extends the antimicrobial effectiveness out to longer time periods.

Conclusions

Bioactive glass particulate possesses short-term antimicrobial properties. While the effect varied depending on the organism tested, it was effective in reducing the levels of all microbes evaluated using a standardized USP test.

References

1. Allan I, Newman H, Wilson M: Antibacterial Activity of Particulate Bioglass® Against Supra- and Subgingival bacteria. *Biomaterials*, 22:1683, 2001.
2. Stoor P, Soderling E, Salonen JI: Antibacterial Effects of a Bioactive Glass Paste on Oral Microorganisms. *Acta Odontol Scand*, 56:161, 1998.

Presented at the Society for Biomaterials 30th Annual Meeting and Exposition, Memphis, TN, April 2005. Transaction #558