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Antimicrobial effectiveness of Prosidyan Fibergraft® bone graft material against a range of microorganisms

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Abstract

Extensive research has documented the clinically interesting antimicrobial activity of 45S5 bioactive glasses, which has been attributed to the exchange of alkaline species between the surface of the glass and the surrounding aqueous environment. The release of alkaline species induces an increase in pH, which inhibits the viability of microbiota. An increase in the surface area of the bioactive glass has been shown to release a greater number of ions in solution, which increases the surrounding pH, resulting in a greater antimicrobial efficacy. Fibergraft® Bone Graft Material is made entirely from crystalline 45S5 bioactive glass, but offers exponentially greater specific surface area than other bioactive glass products. Until now, no previous data has documented the unique ability of Fibergraft® from inhibiting bacterial growth *in vitro*. The highly controlled USP <51> Antimicrobial Effectiveness Test demonstrated considerable antimicrobial activity of Fibergraft® against a range of common microorganisms *in vitro*. Further investigation is required to evidence whether or not this bone graft substitute also exhibits this property *in vivo*.

Introduction

Extensive literature has documented the unique bacterial growth inhibiting effect of 45S5 bioactive glass. The clinically interesting antimicrobial properties of 4S5S bioactive glass can be attributed to the continuous release of alkaline species from the surface of the glass [1]. The release of alkali ions, mainly Na^+, into the aqueous environment induces a slight increase in pH, which is not well-tolerated by microbiota, resulting in the inhibition of bacteria to grow [2,3]. By increasing the surface area, and thus increasing the active exchange surface of glass and its surrounding environment, a substantial increase in the number of ions are released into solution [4]. The greater the number of ions in solution, the greater increase in pH, which results in increased antimicrobial efficacy [5].

Prosidyan's Fibergraft extender is a purely synthetic bone graft substitute made entirely from crystalline 45S5 bioactive glass [6]. In addition to its clinical success in stimulating bone growth and repair, Fibergraft exhibits strong antimicrobial properties in vitro, demonstrated by commercialized antimicrobial effectiveness tests, as shown in Table 1. Inside the granule of each Fibergraft BG Morsel is a nest of fibers, creating vast surface area and pore sizes among the micro and nano-sized fibers and microspheres of bioactive glass [6]. In particular, the concentrated nano-sized fibers inside each granule offer vastly increased surface area than conventional bioactive glass [6]. Fibergraft's powerful bacterial growth inhibiting effect can be attributed to these unique features that provide exponentially greater specific surface area than other products that use only particulates or microspheres, allowing it to release more alkaline species per cubic unit and display stronger antimicrobial properties [7].

The purpose of this analysis was to determine the effect of exposure to Fibergraft BG Morsels on the viability of a range of microorganisms based on the Antimicrobial Effectiveness Test. Throughout history, this particular test has evolved to study a system's ability to protect against microbial contamination during storage and usage of a product [8].

Materials and methods

Fibergraft® Bone Graft Substitute Material was used for one viability study on five different microorganisms that are familiar to today's practitioners [8]. The antimicrobial effectiveness was assessed by a third-party commercial laboratory, Biotest Laboratories, Inc., by using the USP <51> Antimicrobial Effectiveness Test (AET). The AET is designed to demonstrate the ability of a pharmaceutical product to inhibit the growth of a contaminant in the product, commonly referred to as its preservative system [8]. It is important for practitioners to keep in mind that the AET is a laboratory test performed under careful controls and is not intended to be a simulation of real-world clinical situations [8].

Test organisms

The five species that were tested with Fibergraft material were each tested separately. This method was proven to indicate preservative effectiveness more accurately than testing the organisms together [9]. The five microorganisms tested were as follows:

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Staphylococcus aureus ATCC 6538

Escherichia coli ATCC 8739

Pseudomonas aeruginosa ATCC 9027

Candida albicans ATCC 10231

Aspergillus brasiliensis ATCC 16404

Procedure

The product is inoculated with a known quantity of specified microorganisms and the quantity of microorganisms found in the control sample is compared to the sample at Day 0, Day 7, Day 14, and Day 28 [3]. The Antimicrobial Effectiveness Test was performed according to the USP <51> guidelines [8].

Results

Table 1 reveals the approximate population number of microorganism in each colony at Inoculum and after Day 0, Day 7, Day 14, and Day 28. Fibergraft BG Morsels exerted an antimicrobial effect against all five strains that were tested. This effect was greater after increased days of exposure to the product. After Day 7, a lesser antimicrobial effect was observed against aspergillus brasiliensis compared to the other strains.

Table 2 shows the percentage of microorganisms killed at the end of Day 0, Day 7, Day 14, and Day 28 for each strain. Approximately 100% of the microbial population was killed after Day 7 of inoculation in four of the five strains: staphylococcus aureus, escherichia coli, pseudomonas aeruginosa, and candida albicans. It was not until Day 28 after inoculation that the greatest effect was seen in the fifth strain, aspergillus brasiliensis, in which 98.8% was killed. This was the only strain tested that did not exhibit 100% antimicrobial effectiveness after Day 7.

Table 1. Population in colony forming units (CFU)

Population in Colony Forming Units (CFU)					
Organism	Inoculum	Day 0	Day 7	Day 14	Day 28
Staphylococcus aureus (ATCC 6538)	2.7×10^6/1.0 mL	5.9×10^5/ Product	<100/ Product	<100/ Product	<100/ Product
Escherichia coli (ATCC 8739)	2.0×10^6/1.0 mL	5.7×10^5/ Product	<100/ Product	<100/ Product	<100/ Product
Pseudomonas aeruginosa (ATCC 9027)	3.1×10^6/1.0 mL	5.6×10^5/ Product	<100/ Product	< 00/ Product	<100/ Product
Candid albicans (ATCC 10231)	5.0×10^5/1.0 mL	2.1×10^4/ Product	<100/ Product	<100/ Product	<100/ Product
Aspergillus brasiliensis (ATCC 16404)	3.1×10^5/1.0 mL	4.5×10^4/ Product	3.5×10^4/ Product	3.3×10^4/ Product	3.7×10^3/ Product

Table 2. Percentage of microorganisms for each strain

	Percentage Killed			
Organism	Day 0	Day 7	Day 14	Day 28
Staphyococcus aureus (ATCC 6538)	78.1%	~100%	~100%	~100%
Escherichia coli (ATCC 8739)	71.5%	~100%	~100%	~100%
Pseudomonas aeruginosa (ATCC 9027)	81.9%	~100%	~100%	~100%
Candid albicans (ATCC 10231)	95.8%	~100%	~100%	~100%
Aspergillus brasiliensis (ATCC 16404)	85.5%	88.7%	89.4%	98.8%

Conclusions

Fibergraft BG Morsels showed excellent *in vitro* antimicrobial activity against a range of common pathogens. This property has been directly validated by the USP <51> Antimicrobial Effectiveness Test (Table 1). Further investigation is required to determine whether or not Fibergraft Material also exhibits this property *in vivo*.

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