BioActive Glass S53P4 Used in the Treatment of Bone Infections

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One of the main features of modern medicine is the multifactorial approach of pathology resulting in interdisciplinary research focused on complex treatment of diseases, involving multiple branches not only from medicine, but from basic research, too. One of these situations is the vast field of advanced biomaterials, such as bioactive glasses (BAG), which is of great interest for research, industry and medicine, as well. This paper refers to one of the most challenging pattern-that of bone defects resulting from infections, when, so far, the only available types of grafts are BAG due to their combined healing and antibacterial properties.The authors underline the properties and clinical results with BAG-S53P4 in treating post-traumatic osteitis, a frequent and severe complication of skeletal pathology, with tremendous individual and social costs and consequences. The basic structural features are analyzed from the point of view of clinical indications and current results, reflecting their main actions, which make them proper for the most difficult cases of bone infections: osetostimulation, ostoconductivity sf antimicrobial

Keywords: bioactive glasses, osteitis, silicon, ostreoconduction

BioActive Glasses [BAG] are synthetic biocompatible inorganic materials with a controlled ionic release which induce cellular responses at the molecular level [1]. Like any other materials, they bond to the surrounding tissues and induce a particular response from them. Created for the first time in 1971 by Hench and co-workers, the series starts with 45S5, with 45% SiO_2 24-5% Na_2O_2, 24-5% CaO and 6% P_2O_5, later improved by ion substitution [2]. They became increasingly used for covering bone defects due to their demonstrated properties of wound healing, osteoconduction and angiogenesis [3-5].

Along with their osteo-formative action, BAG have been proven to have antibacterial activity, first studied in dental medicine [6], then extended to orthopaedics, especially due to increasing incidence of bone infections, which is nowadays ranging from 1% in primary arthroplasties, to 5% in revisions, with a considerable risk of re-infection, up to 20% [7], the numbers being even higher in trauma ,especially after open fractures (52% after type IIIB open fractures, according to Gustillo) [8].

Bone infections are included in the category of deep infections; they require prolonged treatment and severely affect the socio-professional status of the patient, due to long term disability.

Most of the studies reported different strains of *Staphylococcus* as an etiology for these infections: coagulase-negative, *Methicillin Sensitive St. aureus* (MSSA) and *Methicillin Resistant St. Aureus* (MRSA) [9].

Under these circumstances, interest for the antibacterial properties of BAG grew and research focused on studying the factors influencing their properties.

Their chemical composition strongly influences their activity, especially their bonding to tissues (then determining their absorption and thus their biological impact) which is maximum when the SiO_2 content represents less than 60% in weight; the most rapid tissular bonding is obtained when the SiO_2 represents 45-52% of the weight, when the BAG will strongly connect to the bone and to the soft tissues, as well. Increasing the SiO_2 content to 55– 60% of the weight will react more slowly, thus having a delayed integration, with no soft-tissue binding [10]

The value of *p*H and fluid dynamics have been demonstrated to impact the antibacterial effect for most of them. For example, for BAG 45S5, which acts by increasing the *p*H and the osmotic pressure, a supplementary 'needle-like' sharp mechanically damaging action of the glass debris on the cell wall was described.

Although, under elevated *p*H conditions, 45S5 has no antibacterial effect on *S. aureus*, with a concentration dependent anti *E. Coli* activity, but ceasing when the *p*H is neutralised; this self-limiting mechanism does not become active in vivo because such large *p*H changes are less frequent due to compensating mechanisms[11].

In the class of BAG, the compound named S53P4, has become extensively studied, due to its promising results so far. Regarding its structure, it contains elements naturally existing in the human body, in the following proportions: 53% SiO_2, 23% Na_2O_2, 20% CaO, and 4% P_2O_5 [12], being included into the 4-compounds group of BAG. It is fully resorbable, with low speed, over a period of several years, so as full gap filling is allowed. [12]. Although BAG have been produced as granules, putty or plates, this paper will refer to the granules solely, as they have been intensively studied and the presented cases were treated using granules, as well.

The *in vivo* studies on rabbits revealed interesting data regarding the dynamics of the activity of BAG-S53P4 within the first two weeks, undifferentiated mesenchymal tissue surrounds the BAG and an apparent blocking activity of the BAG upon bone formation was described; then, in 4-8 weeks, abundant formation of immature woven new bone, followed by its fast resorption occurs; in the subsequent period of new bone remodeling, maturation results in lamellar bone, partially covering the microspheres [13].

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Opposite to the nonwage samples, a constant time-related new-bone generation was described, and, proving the high tissular affinity, 35.6–55.8% of the outer perimeter of bioactive glass microsphere was in contact with on grown new bone (affinity index) [13].

From a clinical point of view, two major aspects regarding S53P4 are going to be discussed: its ability to stimulate bone growth and its antibacterial activity.

Bone growth stimulating activity

According to Vallimaki and Aro [13], the presence of silica and CaP reaction layer on the surface of the BAG, as well as a direct contact between this layer and the new generated bone, demonstrated the mechanism of activity of BAG, which resembles in ion leaching at its surface when it makes contact to body fluids:

- a rapid exchange of Na^+ from the glass with H^+ and H_3O^+ from the surrounding tissue, results in silanol formation (SiOH) at the BAG surface, which then is repolymerized, forming a SiO_2- rich layer;
- Ca^{2+} and PO_3^{4-} groups' migration to the surface and crystallization results in a $CaO\text{-}P_2O_5$ hydroxyapatite (HA) layer on top of the Si-rich layer;
- the HA layer has chemotactic activity for growth factors, absorbing them; consequently, the osteoprogenitor cells are stimulated and produce extracellular matrix and subsequently, new bone [13,16].

The chemotactic effect of BAG upon cells is supposed to be produced by:

-stimulating the osteoprogenitor cells to proliferate and differentiate into matrix-producing osteoblasts and enhancing the osteoblastic activity, inclusive by their gene expression;

-stimulating multipotent stromal bone marrow cells functions;

-the Si-rich layer acts as a template for calcium phosphate precipitation;

-the extracellular proteins, especially fibronectin, attract macrophages, and mesenchymal stem cells and osteoprogenitor cells;

-stimulating a rapid bur balanced turn-over- bone-synthesis and resorption; according to Northern, mRNA s are increased for both synthesis (type I, II and III collagens, osteocalcin, osteonectin and osteopontin) and resorption (MMP-9, TRACP, Cathepsin K) [13, 16].

So, basically, three major steps result in BAG bonding and bone generation:

*A.*HA generation- by the intermediate phases:
  i. SiOH;
  ii. Si_2O – by polycondensation of SiOH;
  iii. Adsorption of $Ca + PO_4 + CO_3$ amorphous;
  iv. HA crystallization;

*B.*cellularisation of HA layer- macrophages, stem mesenchymal cells, differentiated mesenchymal cells;

C.matrix formation-production and crystallization [13].

These properties represent the premises for the Bone growth stimulating activity of S53P4 which consists of the following:

- osteoconductive activity consists of creating a 3D scaffold on which osteoblasts can migrate and build new bone;

- osteostimulative (not osteoinductive) - refers to the ability to activate genes responsible for bone formation in osteogenic cells;

- angiogenesis -stimulate release of angiogenetic growth factors, promote angiogenesis by enhancing the tubular networks in a fibroblastic environment, thus supporting cellular proliferation and migration, including for the endothelial cells, vessel branching and anastomosis [13].

Antibacterial activity

The ability of S53P4 to inhibit bacterial growthresults from ion release from the surface of the granules, as shown:

- releasing of sodium, which increases the *p*H, disturbing the bacterial cycle; for S53P4, the *p*H max is 11.65;

- releasing of Na, Ca, P and Si, which increase the osmotic pressure, thus creating a hostile environment for the bacteria [17-19].

The antibacterial activity depends on the dimensions of the granules; as presented in a study published in 2014, Coraça -Huber and his team studied the effectiveness of different sizes of BAG- S53P4 against St. Aureus biofilms grown on titanium discs in vitro. Two different types of granules: 0.5-0.8 mm and <45 μm were placed in contact with biofilm-containing disks, with inert glass beads as control group. The number of CFU (Colony forming units) was used as indicator the ability to suppress biofilm formation. The measurements confirmed that BAG-S53P4 can clearly suppress *S. aureus* biofilm formation, and the suppression rate was significantly higher for the granules smaller than 45 μm than for those sized 0.5-0.8 mm [20].

A unique feature for this BAG is the activity on the biofilm; according to recently published research, apart from its osteoconductive and antimicrobial properties, BAG-S53P4 is active on biofilm, too; titanium discs covered with *Multiresistant Staphylococcus epidermidis, Acinetobacter baumannii* and *Klebsiella pneumoniae* isolated from bone and joint infections where incubated with inert glass (control group) and BAG-S53-P4 (study group); using confocal laser scanning microscopy, it was demonstrated that the volume of biomass was considerably lower in the study group, thus proving reduced biofilm production. Added to the increased percentage of dead cells in the BAG group, it was concluded that these demonstrated antibacterial activity for S53P4 [21-22].

Experimental part

Material and methods

BioActive Glass S53P4 was purchased from BonAlive Biomaterials Ltd company. To illustrate the clinical implications of the above described properties of BAG-S53P4, in the present study 4 cases with bone infections were analyzed, in which the above mentioned product was used as a bone graft.

Mean age of the patients was 35 years.(22-52 yrs.) and mean time between trauma and the onset of infection was 3 months.

The infection was situated at the level of the femur (3 cases) and calcaneus (1 case), in all the cases following trauma (fractures). The etiology was *St. Aureus* in 3 cases and *Ps. aeruginosa* in 1 case.

In all the cases, the protocol was represented by the following steps:

- positive microbiological diagnosis- from tissular samples, swabs and sonication;

- surgical treatment: Implant removal (sonication) thorough repeated debridement, followed by external fixation; soft tissue coverage;

- bone grafting with BonAlive; due to the fact that all the chemical properties of BAG become active in an aqueous environment, the granules must be conditioned with sterile saline solutions before application;

- conversion to internal fixation with additional soft tissue coverage;

- thrombo-prophylaxis with Low Molecular Weight Heparins according to the risk factors of the patient;

- antibiotics according to the microbiological evaluation;

- clinical and radiological follow up after 3, 6 and 12 months after surgery.

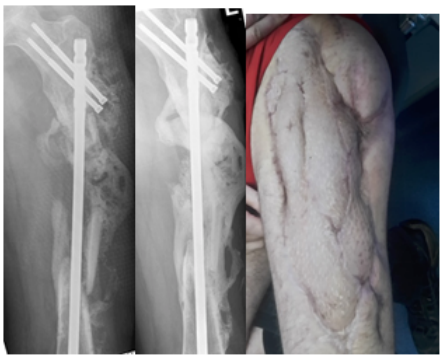
In all the cases, the infection was cured; clinical and radiological healing was obtained in all the cases; no infection relapse was noted; inflammatory tests were constantly monitored, with no significant increase.

From this group, in one case, this product was used in a large bone defect, following an acute post-traumatic infection. The patient was admitted in our hospital 1 week after the initial crushing trauma, a high energy injury with comminuted IIIB open fracture of the femoral shaft, infected with *Acinetobacter baumanii*. Besides the clinical aspect with considerable necrotic soft tissue, the X-ray revealed a large bone defect in the middle third of the femur, temporarily stabilized with an external fixator (ExFix). After repeated debridements, the fracture site was finally covered with vital soft tissues.

Cutaneous grafts were used for covering the defect, then the external fixator was converted to an intramedullary nail ; due to the large bone defect affecting the internal middle third of the femoral cortices, grafting was indicated; since the large amount could not be provided by autograft and the chances of infection relapse were considerable, BAG were taken into consideration because of their properties.

BonAlive granules were administered percutaneously, through an incision on the anterior aspect of the thigh, in healthy tissue, partially covering the bone gap.

Due to the ostestimulative properties, the BAG induced bone formation, so, progressive decrease of the bone gap is obvious (fig. 1).



a *b* *c*

Fig. 1. Aspect before BAG (*a*) and 8 months after with optimal bone integration and bone growth (*b*); complete healing of the soft tissues (*c*)

The particular aspect of this case is related to the massive bone deffect, which could have been covered by a combined autograft+bone substitute unless it was infected. The presence of infection completely changes the perspective, since no bone substitute except the BAG can be used in septic environments. Therefore, the method of introducing (by a minimal invaseive procedure) the BonAlive granules acted both as a bone growth–stimulating material and as an antibacterial agent, as well.

Results and discussions

The main issue underlined by this work is represented by the properties of BAG-S53P4 which makes it suitable for solving the particular problem of treating bone infections due to its antibacterial activity and bone-stimulating properties.

Two main characteristics must be underlined for bone infections, in order to properly approach their treatment: Firstly, the area of the bone chronically infected, the "sequestrum"; is a piece of necrotic bone, avascular, thus impenetrable to any antibiotic. Secondly, in the presence of any implant, the bacteria organise on its surface as a biofilm; this is a complex network, within which the microorganisms live and divide, being totally protected from any systemic antibiotic [23-24].

That is why treatment must start with implant removal and continued with complete and thorough excision of the infected bone and soft tissue, so a bone defect occurs, requiring replacement. Different types of bone grafts or substitutes can be used in the absence of infection, but septic complications require complicate surgical procedures. Local or free flats, as well as bone transport were used for replacing the bone, since in the presence of infection any simple graft will fail. Due to poor vascularity, local delivery of antibiotics replaced the systemic one, using different vehicles; although still widely used the antibiotic-loaded polymethacrilate (PMMA) beads have limitations as they require secondary surgery for their removal [25]. Research also focused on incorporating different substances with antimicrobial properties , with promising results after in vitro testing , waiting for further in vivo studies [26].

Therefore, BAG remain, until now, the only alternative for local antimicrobial activity (as PMMA) without needing removal, acting in the same time as a bone substitute, fulfilling all the pre-requisites of the *ideal* bone substitute, as they are: biocompatible, bioresorbable, osteo-conductive, osteostimulative (although not osteoinductive) structurally similar to bone form a biological and mechanical point of view, easy to use, cost-effective, safe and easy handling.

As the clinical results are promising, BonAlive®granules is the only bone graft substitute for which the claim of inhibition of bacterial growth has been approved and its indication for treating chronic osteitis is clear.

Several problems still need *further research:* due to the tendency to crystallization during manufacturing into different shapes, at high temperature of this group, more complex compounds, the so-called *modified systems*, based on Na_2O-K_2O-MgO-CaO-B_2O_3 -P_2O_5 -SiO_2 [27], have been produced., thus giving the possibility to manufacture BAG into microspheres, fibers and porous implants, clinically more advantageous; in the same time, loading bone grafts or bone substitutes with antibiotics is more and more studied, and even the problem of enhancing BAG with antibiotic has been taken into consideration [28, 29].

Resulting from the data published so far, BAG in general, and S53P4 (BonAlive) in particular have proven to be a valuable option in the treatment osteomyelitis [30]. It is still to be mentioned that regardless the outstanding properties of the BAGs, their utility has not been studied in the absence of surgical debridement, which is unanimously considered as the starting point of the treatment.

Conclusions

Treating bone infections require a special algorithm, in which surgical debridement is mandatory, with bone excision up to the unaffected area. Covering the bone defect under these circumstances is challanging, since bone grafting in a septic environment will definitely fail; on the other hand, local antibiotic delivery is impaired, so the key requirememnt is for a material able to bond to the local tissues, promote bone growth and act as an antibacterial agent. None of the currently biomaterials used as bone substitutes fulfill these requirememnts except for the BioActive Glasses, which perform both of these tasks. Therefore, one of the BAGs, S53P4 has been extensively studied, resulting the conclusion that it is able

to cure osteitis. Its structure and chemical properties provides eficacy and safety, thus drawing the attention of the orthopaedic surgeons (and not only theirs) upon the properties of BioActiveGlasses, and rising the opportunities for future research.

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Manuscript received: 23.11.2016