PRECLINICAL and CLINICAL STUDIES on BIOACTIVE GLASS and LEONARDO – Oral surgery
Hydroxyapatite is hardly soluble and its in vivo degradation rate is ly degrade while being replaced by the natural host bone tissue. Absorbable biomaterials are known for their ability to gradually degrade of bioactive glass happens, followed by numerous and complex reactions. Figure 1 below summarizes the cascade of the 11 reactions occurring at the surface of bioactive glass during the formation of a solid grip with the bone.

The five first reactions take place in the periphery of bioactive glass and do not depend on the presence of tissue. This reactions has been studied and could take place in distilled water or buffer solution like TRIS or SBF. These reactions make up a layer of crystallized carbon hydroxyapatite at the top of material. The implant fastens down with the tissue for the step 6 to 11 like as growth of bone.

3. Osteostimulation

Recent in vitro studies have been led to the understanding of the mechanism of action of bioactive glass10,11. They show that inorganic products such as silicon and calcium have an influence on the stem cells differentiation and on the growth of bone. Besides, the bone mineralization happens thanks to phosphor transit. The dissolution products of bioactive glass 45S5 develop an extracellular environ
tment that is capable of supporting osteoblast phenotype expression and extracellular matrix deposition and mineralization in vitro12. The five first reactions take place in the periphery of bioactive glass and do not depend on the presence of tissue. This reactions has been studied and could take place in distilled water or buffer solution like TRIS or SBF. These reactions make up a layer of crystallized carbon hydroxyapatite at the top of material. The implant fastens down with the tissue for the step 6 to 11 like as growth of bone.

BIOCOMPATIBILITY TESTS PERFORMED

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<td>PROLIFERATION AND GROWTH OF BONE</td>
<td>Irritation (ISO 10993-10)</td>
</tr>
<tr>
<td>CRYSTALLIZATION OF MATRIX</td>
<td>Delayed-type hypersensitivity (ISO 10993-10)</td>
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<td>GENERATION OF MATRIX</td>
<td>Acute systemic toxicity (ISO 10993-11)</td>
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<td>DIFFERENTIATION OF STEM CELLS</td>
<td>Ames test (ISO 10993-3)</td>
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<td>ATTACHMENT OF STEM CELLS</td>
<td>Pyrogenicity (USP 31)</td>
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<td>ACTION OF MACROPHAGES</td>
<td>Cytotoxicity (USP 32)</td>
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<td>ADSORPTION OF BIOLOGICAL MOIETIES IN HCA LAYER</td>
<td></td>
</tr>
<tr>
<td>CRYSTALLIZATION OF HYDROXY CARBONATE APATITE (HCA)</td>
<td></td>
</tr>
<tr>
<td>ADSORPTION OF ANOMPROP (2P3O105)</td>
<td></td>
</tr>
<tr>
<td>POLYCONDENSATION OF SiOH + SiOH</td>
<td></td>
</tr>
<tr>
<td>FORMATION OF SOF+ BONDS</td>
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<tr>
<td>BIOACTIVE GLASS</td>
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The set of biocompatibility tests performed show that bioactive glass 45S5 products:

- are not cytotoxic
- do not induce irritation
- do not induce delayed-type hypersensitivity
- do not induce acute systemic toxicity
- are neither toxic nor mutagen
- are not pyrogenic

4. Biocompatibility evaluation

Biocompatibility evaluation has been conducted following the table of the NF EN ISO 10993-1 standard. Since bioactive glass is a well-known material which has been clinically used for more than 20 years, biocompatibility tests which have been conducted aim at evaluating the toxicity of manufacturing residues.

Here is the list of the tests which have been conducted and the providers involved in the study:
4.2 Implantation of different bone grafting materials on rabbits: global evaluation of NORAKER products

An implantation study has been conducted on rabbits in order to evaluate the biological response of tissues to different bone grafting materials, including bioactive glass 45S5. This study has been conducted in adaptation to the ISO 10993-6 norm. Bioactive glass 45S5 product is compared to a ß-TCP control material. Granules are implanted in 5 animals for each time, in the area of the femoral epiphysis (either distal or proximal). Observation times are 4, 12 and 24 weeks.

The picture shows a normal marrow without any sign of residual ossification. Some Bioactive glass 45S5 residues can be observed, under granulated shapes (BG) in very isolated trabeculae. After 12 weeks, Bioactive glass 45S5 granules quickly resorbed whereas control material (ß-TCP) had a slower degradation. Outside the cortical area, the picture shows a heap of bioactive glass 45S5 particulates. They are included in a soft conjonctive tissue which is denser at the interface with particulates. In the medullar implantation area, some trabeculae remain. They seem to result from the presence of the particulates.

Bioactive glass 45S5 granules (material) are of particle shapes. We can see numerous monocyte cells in the existing soft connective tissue, between the ossified areas, only at the surface of the particles (dark blue).

After 4 weeks, at histological observation, both materials (bioactive glass 45S5 and control) have a bioactive appearance. They went through different successive stages: 1/ invasion by monocyte cells, 2/ by a soft connective tissue, 3/ osteoblasts differentiation at the surface of the material, 4/ degradation of the material (with different rates), and replacement by mature bone.

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Bioactive glass 45S5 and ß-TCP materials can be considered as bioactive implants which contribute bone remodeling. At first, they are colonized by monocyte cells, then by a soft conjonctive tissue. Osteoblasts then differentiate at their surface and are responsible for the synthesis of immature bone matrix. Finally, the immature matrix is remodelled, replaced by mature bone. During this final stage, implants are damaged with variable-speed. 12 weeks the material implantation, bioactive glass 45S5 was more damaged than the ß-TCP control material (-TCP). After 24 weeks, both materials were damaged and totally integrated to the bone. No foreign body was visible in the implantation sites.

Medical device vigilance has been conducted on bioactive glass products available in the market. One alert has been had as a consequence a hospitalization of patient despite the relationship between inflammation and Perioglas use was established.
## 2. Publications on bioactive glass

Only the clinical studies with more than 12 patients have been selected. They can be found in the table below, named [EC1] to [EC16].

<table>
<thead>
<tr>
<th>Title</th>
<th>Authors</th>
<th>Journal</th>
<th>Indication</th>
<th>Patients</th>
<th>Follow-up</th>
<th>Conclusion</th>
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<tr>
<td>Using 45S5 Bioglass cones as endosseous ridge maintenance implants to prevent alveolar ridge resorption: a 5-year evaluation [EC2]</td>
<td>Stanley et al.</td>
<td>J Periodontol (72) [90]</td>
<td>Use of bioactive glass 45S5 cones to delay the reformation of alveolar ridges.</td>
<td>186 implants in 20 recalled patients</td>
<td>Clinical follow up at five-years.</td>
<td>Good results. Only 7.7% of the implants required removal. High rate of bioactive glass core retention 89.7% after 5 years. The authors recommend their placement into fresh sockets to maintain the alveolar ridge.</td>
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<tr>
<td>Effects of pretreatment clinical parameters on bioactive glass implantation in intrabony periodontal defects [EC3]</td>
<td>Park et al.</td>
<td>J Periodontol (72) [125]</td>
<td>Clinical effects of bioactive glass 45S5 implantation in intrabony periodontal defects.</td>
<td>38 intrabony defects from 38 patients</td>
<td>Evaluation of proliferative and postoperative probing depth (PD), clinical attachment level (CAL), bone probing depth (BPD) and gingival recession at 6 months after surgery.</td>
<td>Use of a bone substitute in a flap operation resulted in significantly greater improvements in CAL and BPD over flap operation alone and seemed to have positive effects in postoperative PD, CAL, BPD in those cases with more severe proliferative CAL and BPD. Equal clinical results with bioactive glass bone replacement graft material and e-PTFE barriers in mandibular molar class II furcations. Bioactive glass required no additional material removal procedures.</td>
</tr>
<tr>
<td>Clinical comparison of bioactive glass bone replacement graft material and expanded polytetrafluoroethylene barrier membrane in treating human maxillomalar maxillary class II furcations [EC4]</td>
<td>Yaliva et al.</td>
<td>J Periodontol (72) [125]</td>
<td>Treatment problems of mandibular Class II furcations.</td>
<td>27 pairs of mandibular molars in 27 patients (27 molars with Perioglas + Emdogain from 0.09 to 0.71 mm) and 27 molars with ePTFE membrane</td>
<td>Follow-up during 6 months; clinical attachment level; bone probing depth and gingival recession</td>
<td>Similar results between two groups. Bioactive glass granules can be used together with autogenous bone chips for sinus augmentation procedure; thus decreasing the amount of bone needed.</td>
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</table>

### Bioactive glass and bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis: results of a 5-year clinical and radiological study [EC5]

**Title**: Repair of orbital floor fractures with bioactive glass implants

**Authors**: Ayala et al.

**Journal**: Ophthal Surg

**Indication**: Clinical comparison of bioactive glass bone replacement graft material and expanded polytetrafluoroethylene barrier membrane in treating human maxillomalar maxillary class II furcations [EC4]

**Patients**: 30 patients from 1995 to 1999

**Follow-up**: Clinical and radiological follow-up of 28 patients at one-year

**Conclusion**: Bioactive glass implants were well-integrated. No foreign body reaction and no inflammation. Bone formation around the implants.
Title: Particular bioglass as a grafting material in the treatment of periodontal intra-bony defects [EC14]

Authors: Zamet et al.


Indication: Treatment of periodontal intra-bony defects

Patients: 20 patients: 44 sites (22 sites with Perioglas and 22 sites with sinus floor surgery)

Follow-up: Follow-up was the carried out yearly and at 1, 2, 3, 5 years and 10 years post-surgery. Recording of probing pocket depth, probing attachment level and gingival recession. Radiographic evaluation.

Conclusion: Bioactive glass is as effective as an adjunct to conventional surgery in the treatment of intra-bony defects.

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Title: Four-year results of a prospective-controlled clinical study evaluating healing of intra-bony defects following treatment with an enamel matrix protein-derivative alone or combined with a bioactive glass [EC14].

Authors: Yilmaz et al.


Indication: Regenerative periodontal surgery for furcation defects

Patients: 15 patients treated with bioactive glass + PRP and 22 flaps with Perioglas and 27 with flap surgery

Follow-up: Clinical Follow-up at 4, 6 and 12 months, evaluation of probing depth, probing attachment level and gingival recession

Conclusion: Bioactive glass showed significant improvements in clinical parameters compared to open flap debridement.

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Conclusion: Bioactive glass is as effective as an adjunct to conventional surgery in the treatment of intra-bony defects.
3. Evaluation of the clinical data
Evaluation of clinical publications on bioactive glass is based on the literature review already published. The previous table sums up scientific studies where bioactive glass is used in oral clinical surgeries. This paragraph is devised in two parts:

■ Preclinical and clinical studies on bioactive glass resorption
■ Clinical studies in oral surgery.

3.1 Bioactive glass resorption
Bioactive glass particles are composed of an amorphous mixture of calcium, silicon, phosphate and sodium. The resorption by ionic dissolution improves the contact surface of the material, allowing a better blood circulation and increasing the cells anchoring.

Different in vivo studies assessed bioactive glass resorption. A histomorphometrical study conducted on rabbits showed the dissolution capacities of three different types of bioactive glasses. The authors observed that 45S5 bioactive glass had the most important kinetics of bone fixation and the faster resorption rate. Oonishi’s study showed that the major part of bioactive glass particles is absorbed between 8 to 12 months after implantation in femoral condyles of rabbits. Particles could not be identified at further times of observation.

Ducheyne evaluated the elimination of bioactive glass resorption products and showed that silicon oxide elimination was included into the acceptable limits for the tested animals. The approximate time of silicon oxide elimination is 2.4 mg/day and 100% of implanted silicon oxide is eliminated after 19 days. Another study concerning human beings follow-up has been studying the rate of silicon in the blood. 25 patients had graft surgery using bioactive glass or autologous bone after tumors resection. Blood analyses have been realized at different times until 36 months. Results showed that there is no significant difference in the patients’ blood silicon concentration between the group treated with bioactive glass only and the group treated with autologous bone. Besides, the quantity of implanted bioactive glass, which depends on the volume of the defect to fill, has no influence on the silicon concentration in blood.

Moreover, studies have proved that silicon is involved in bone remodelling. Indeed, Reffitt has showed that addition of orthosilicic acid in osteoblast cell culture stimulated type I collagen synthesis and osteoblastic differentiation. We can think that silicon released by bioactive glass could have a positive effect on bone remodelling.

3.2 Clinical studies in oral surgery
There are numerous clinical studies related to the use of bioactive glass in oral surgery, with a follow-up at different levels: clinical parameters, radiographs, biopsies. The main clinical studies in previous table deal with the treatment of periodontal defects.

Diagnostic of periodontal diseases is made on clinical signs such as redness, oedema or inflammation. Periodontal clinical examination has to evaluate presence and quantity of bacterium blotch, bleeding, probe depth, probe attachment level and mobility. Three different surgical techniques have been identified: flap surgery, guided tissue regeneration technique, and bone grafting treatment.

Three comparative and randomized studies compare two first techniques to bone grafting using bioactive glass. Zamet showed that bone grafting with bioactive glass is better than flap surgery from bone remodelling aspect. Froum and Park showed that bone grafting with bioactive glass is the best: the probing depth was better and attachment level too.

3.3 Literature review conclusion
Clinical data showed that the use of bioactive glass for bone grafting in oral surgery gives good results, similarly to autologous bone. The majority of these published studies are randomized and comparative studies with a little number of patients. A clinical study with more patients could improve the scientific evidence of effectiveness of bioactive glass.

This great number of studies proves that the use of bioactive glass as a bone grafting material is safe and gives good results that are similar to the use of autologous bone.

This evaluation of clinical studies is completed with the clinical follow-up of Bioactive glass 45S5 product.

[10 11]