Comparison in *in-vivo* response between a bioactive glass and a non-bioactive glass

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The authors report on the *in-vivo* comparison, in the rabbit, between the response to a bioactive glass and the response to a non-bioactive glass. Implants have been performed in muscle and bone. Two different glasses were investigated, namely B01 and I02. B01 is a glass designed to be degradable and resorbable and has a percentual molar composition of: SiO_2 49.6%; P_2O_5 2.7%; $CaO + MgO + Na_2O + K_2O + Al_2O_3$ 47.7% with a 1 : 1 CaO/Na_2O ratio. I02 is a sodium-calcium-silicate non-resorbable glass lacking P_2O_5 and has a percentual molar composition of: SiO_2 70.7%; $CaO + MgO + Na_2O + K_2O + Al_2O_3$ 29.3%. *In-vivo* tests were planned as: (a) intramuscular implants of glass cylinders in the *rectus femoris* and retrievals took place at 2, 16 and 43 weeks; (b) intraosseus implants of glass cylinders in the distal femural canal and retrievals took place at 8 and 43 weeks. Histology and light microscopy analysis followed. Bioactive degradable glass elicits a favorable response both in muscle and bone; a gradual degradation process leads to disruption and partial resorption of the material and a tight apposition is promoted with the newly formed bone. The non-bioactive sodium-calcium-silicate glass (named I02) may elicit, like the bioactive degradable B01, a favorable response which is characterized by the absence of inflammatory or other adverse reactions; anyway it does not change its structure at an optical microscopic level and it does not promote any tight apposition with bone.
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1. Introduction

A bioactive glass suitable for the coating of metallic substrates has been described previously [1]. Preliminary clinical experience in humans has been carried out by performing total hip replacements with bioactive-glass-coated prosthesis [2, 3]; the longest follow-up has now reached seven years [4]. Degradable bioactive glass coatings are needed since the rationale of the coating is to lead the bone gradually towards the metal. This process should happen without the production, at its end, of bulky non-degradable particles as those observed with the fragmentation of the crystalline phase of hydroxyapatite coatings [5]. The gradual replacement of bioactive-glass-coating by newly-formed bone [6] seems to be clinically related with a better long-term outcome of the implant and a decrease in early 6 months thigh pain [4].

An extensive experimental research accompanies clinical applications; physico-chemical characterization and *in-vitro* studies has been published in the past [1, 7]. *In-vivo* experiments have described the response towards bioactive glass, both as a bulk material and as a coating, in comparison with other materials like titanium alloy and hydroxyapatite [6, 8].

In this paper the authors report on the *in-vivo* comparison, in the rabbit, between the response to a bioactive glass and the response to a non-bioactive glass, both used as a bulk material. Implants have been performed in muscle and bone.

2. Materials and methods

2.1. Glass preparation

Two different glasses were investigated, namely B01 and I02. B01 is a glass designed to be degradable and resorbable and has a percentual molar composition of: SiO_2 49.6%; P_2O_5 2.7%; $CaO + MgO + Na_2O + K_2O + Al_2O_3$ 47.7% with a 1:1 CaO/Na_2O ratio. Preparations respected the following range: Na_2O (7–24%); K_2O (0.5–6%); CaO (8–42%); MgO (1–3%); Al_2O_3 (0.1–2%).

I02 is a sodium-calcium-silicate glass lacking P_2O_5; it is classified as an inert, biocompatible, non-resorbable material. It has a percentual molar composition of: SiO_2 70.7%; $CaO + MgO + Na_2O + K_2O + Al_2O_3$ 29.3%. In solution, I02 releases ten times less SiO_2 and Na_2O than B01 after 96 h.

Fusion of the glass was obtained at 1400 °C. Cylindrical samples were obtained after a new heating at 800 °C.

2.2. Animal model

In-vivo tests were planned as follows: (a) intramuscular implants of glass cylinders; (b) intraosseus implants of glass cylinders. The scheme of the implantation is shown in Table I.

Young adult New Zealand White rabbits, weighing about 2700 g, without preference of sex, were selected as the animal model. Unoperated controls and ''sham-operated'' controls, where the surgical procedure is performed but no cylinder is actually implanted, were included in the implantation protocol.

Material under test was shaped as cylinders of 15×1 mm (length $\times$ diameter). Cylinders were sterilized by gamma rays and single-packaged in sterile envelopes.

Anesthesia was obtained by administration of intramuscular Valium (5 mg/kg), intramuscular Ketalar (50 mg/kg) and subcutaneous Xilocaine. Antibiotic prophylaxis was based on administration of intramuscular Rifocin (250 mg) daily.

To retrieve and process the samples, the rabbit was placed in a special sealed chamber where the atmosphere was quickly saturated with carbon dioxide and left there for 3 min.

2.3. Protocol in muscle

The selected site was the *Vastus Lateralis* and/or *Rectus Femoris* muscle of the thigh. An incision of 2 mm was made distally then a cylinder was smoothly inserted cranially along the direction of the muscular fibers.

Retrievals took place at 2, 16 and 43 weeks (1/2, 4 and 10 months). In half of the samples the cylinder was slipped-off and the muscle placed in 10% formalin. Then followed cryomicrotomy and hematoxilin and eosin staining. Samples were then analyzed by light microscopy.

The second half of the samples were processed like hard tissues, with the cylinder left in place. After seriate passages in ethyl alcohol and methacrylate embedding, sections of 100 microns thickness were analyzed by polarized light microscopy.

TABLE I

Intramuscular implants				
at 2 weeks (0.5 months)				
	01	07ERO13 I02	02	08EIO15 I02
	03	07EIO14 I02	04	08ERO16 I02
	05	01ERO01 B01	06	02EIO03 B01
	07	01EIO02 B01	08	02ERO04 B01
at 16 weeks (4 months)				
	09	03EIX09 I02	10	03EIX10 I02
	11	04EIX05 B01	12	04EIX06 B01
at 43 weeks (10 months)				
	13	05PIO11 I02	14	05PIO12 I02
	15	06PXO07 B01	16	06PIO08 B01
Intraosseous implants				
at 8 weeks (2 months)				
	17	11PBY17 I02	18	11PBY18 I02
	19	10PBY21 I02	20	10PBY22 I02
	21	09PBY19 B01	22	09PBY20 B01
	23	12PBY23 B01	24	12PBY24 B01
at 43 weeks (10 months)				
	25	13PBY25 I02	26	13PBY26 I02
	29	15PBY29 I02	30	15PBY30 I02
	27	14PBY27 B01	28	14PBY28 B01
	31	16PBY31 B01	32	16PBY32 B01

2.4. Protocol in bone

The selected site was the distal femural canal (meta-epiphyseal region). A hole was drilled from the intercondylar groove after access was gained to the articular cavity of the knee by a lateral parapatellar approach. Then a cylinder was inserted along the main axis of the femur. Retrievals took place at 8 and 43 weeks (2 and 10 months).

The retrieved femur was carefully dissected and then placed in 70% ethyl alcohol. The femur was squared in the distal portion; dehydrated in serial passages in alcohol and embedded in methyl-methacrylate. After methacrylate embedding, sections of 100 microns thickness were taken with a rotating diamond-saw microtome; this special saw reduces artifacts due to blade vibrations. Analysis by polarized light microscopy then followed.

Morphological analysis investigates the biological response to implantation procedure, bone growth towards the material and possible resorption of the material.

3. Results

3.1. Response in muscle to non-bioactive glass

Inert non-bioactive and non-degradable I02 glass did not produce any adverse inflammatory response in muscle at any time. It elicits a confinement reaction characterized by a rim of fibrous tissue, always less then 10 cellular rows, in the absence of inflammatory response (Fig. 1). Implant morphology and inner structure of the glass are not morphologically altered.

3.2. Response in muscle to bioactive glass

Bioactive degradable B01 glass elicits a similar response as I02 (Figs 2 and 3). Anyway the inner structure of the glass is morphologycally disrupted by the formation of an outer cortex (Fig. 4) which, macroscopically, consists of a jelly-like substance.

3.3. Response in bone to inert glass

Response to inert non-bioactive non-degradable I02 glass elicits, at all times, a good bone growth; physiological remodeling does not seem to be affected (Fig. 5). A circumferential growth around the implant was seen; there is no tight apposition between bone and glass (Figs 6 and 7). Implant morphology and inner structure of the glass are not altered (Fig. 8).

3.4. Response in bone to bioactive glass

Response to bioactive degradable B01 glass elicits a very good response since bone remodeling proceeds even at the interface with bioactive glass. A tight apposition,

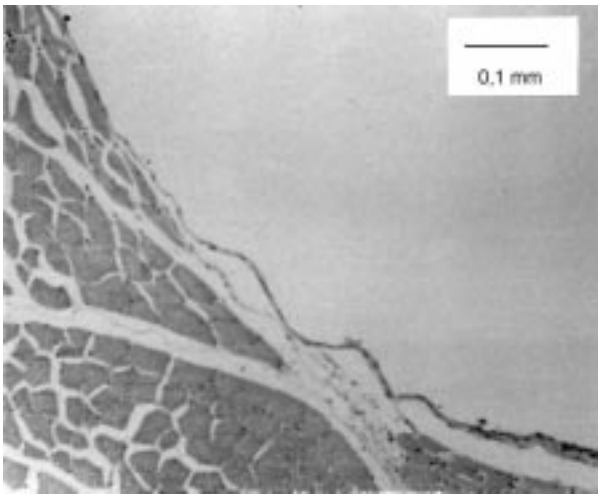

Figure 1 Non-bioactive and non-degradable I02 glass did not produce, in muscle, any adverse inflammatory response (transverse section, H&E stain, 10 ×, at 2 weeks).

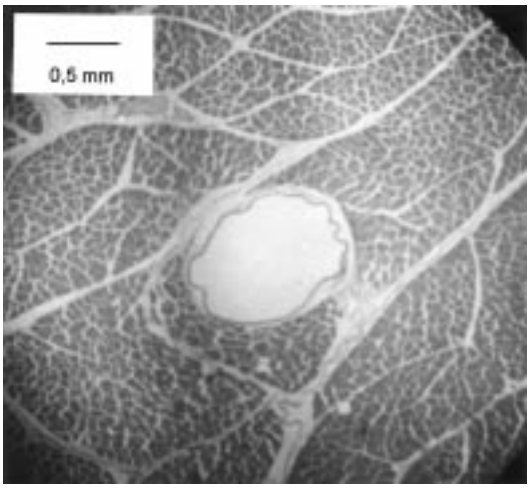

Figure 2 Bioactive degradable B01 glass elicits, in muscle, a similar response to I02 since there is no adverse inflammatory response (transverse section, H&E stain, 4 ×, at 2 weeks).

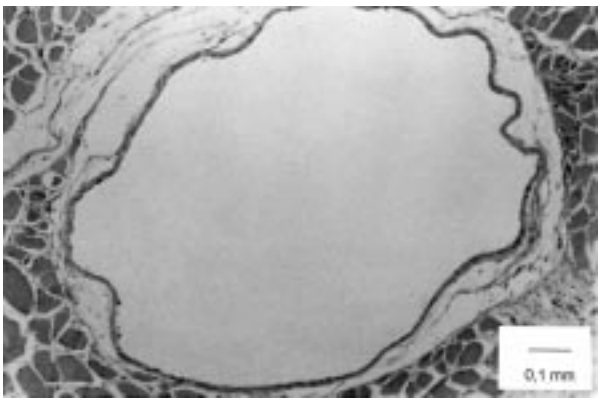

Figure 3 Bioactive degradable B01 glass elicits, in muscle, just a confinement reaction characterized by a rim of fibrous tissue, always less then 10 cellular rows (transverse section, H&E stain, 10 ×, at 16 weeks).

without any fibrous interposition, is observed between bone and glass (Fig. 9).

Similar to implants in muscle, the inner structure of implants in bone is affected by the formation of an outer jelly-like cortex (Fig. 10). Apparently the outer cortex

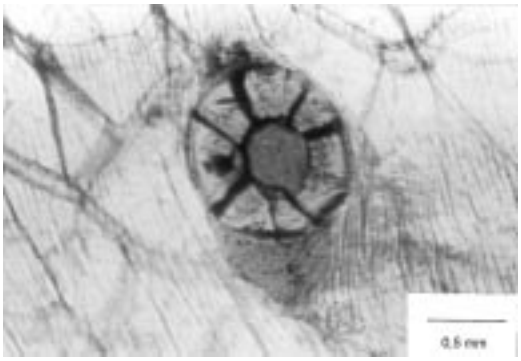

Figure 4 The inner structure of bioactive degradable B01 glass is morphologically disrupted, in muscle, by the formation of an outer cortex which, macroscopically, consists of a jelly-like substance (transverse section, polarized light microscopy, 4 ×, at 43 weeks).

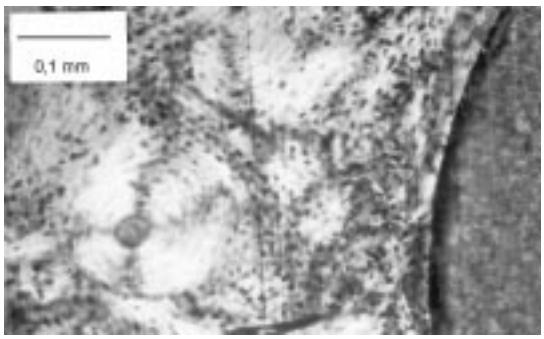

Figure 5 Non-bioactive non-degradable I02 glass elicits, at all times, a good bone growth; physiological remodeling does not seem to be affected (transverse section, polarized light microscopy, 20 ×, at 43 weeks).

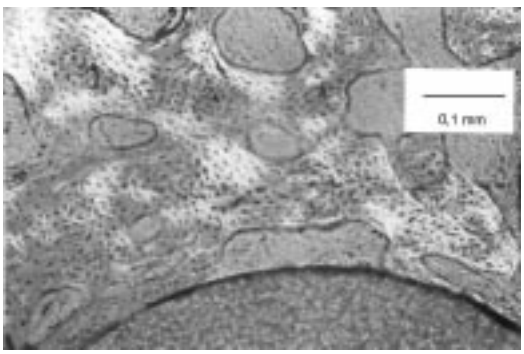

Figure 6 With non-bioactive non-degradable I02 glass, a circumferential growth around the implant is seen (transverse section, polarized light microscopy, 10 ×, at 8 weeks).

favors in the long-term, the gradual disruption of the specimen and its replacement in volume by newly formed bone; anyway remnants of the implant are still present in retrievals at 43 weeks (10 months).

4. Discussion and conclusions

Since the early work of Hench it has been appreciated that a particular composition of glass may be ''bioactive'' and, eventually, promote the growth of newly formed bone in tight apposition with the glass itself [9]. This characteristic is retained when a bioactive glass is

used as a plasma-sprayed coating on a titanium alloy metallic substrate [1].

The exchange of ions between the glass and the biological environment is involved in the mechanism of the bioactive behavior and has been described in several steps by Hench and Andersson [10]. Anyway, other glass compositions (or virtually any glass composition) may promote an ion-exchange in a provided suitable reactive environment but this does not, necessarily, mean that a ''bioactive'' behavior will ensue.

In this paper the authors demonstrate that a common non-bioactive sodium-calcium-silicate glass (named I02) may elicit a favorable response when implanted in muscle and bone. This response is characterized by the absence of inflammatory or other adverse reactions. Two very peculiar aspects of the behavior of bioactive glasses are missing: (a) a non-bioactive glass does not change its structure at an optical microscopic level; (b) a non-bioactive glass does not promote a tight apposition with the newly formed bone.

In conclusion, only bioactive degradable glass elicits a favorable response, both in muscle and bone, which is characterized by a gradual degradation process that leads to the disruption and partial resorption of the material. When this happens in bone, the newly formed trabeculae tightly appose to the glass surface and partially replace in volume the already degraded implant.

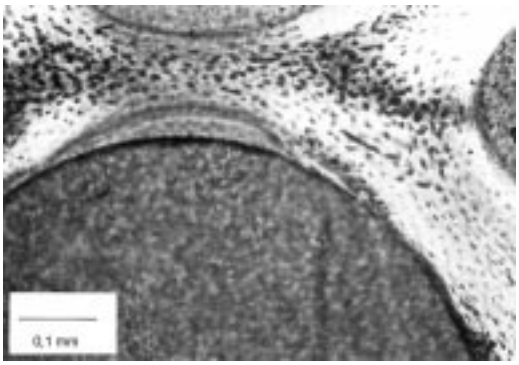

Figure 7 With non-bioactive non-degradable I02 glass, there is no tight apposition between bone and glass (transverse section, polarized light microscopy, 20 ×, at 43 weeks).

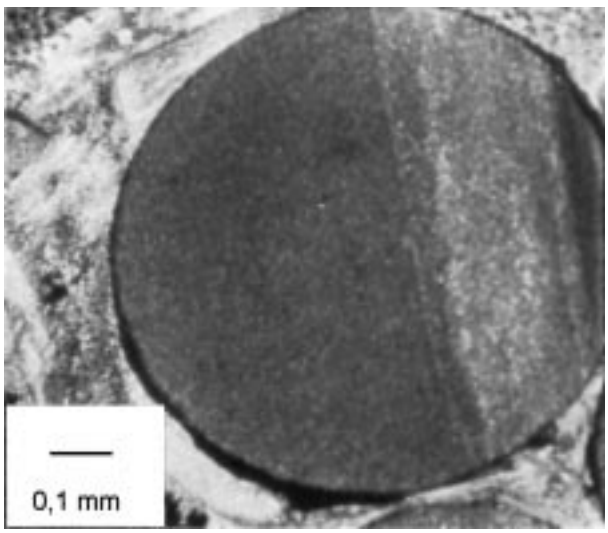

Figure 8 With non-bioactive non-degradable I02 glass, implant morphology and inner structure of the glass are not altered (transverse section, polarized light microscopy, 4 ×, at 43 weeks).

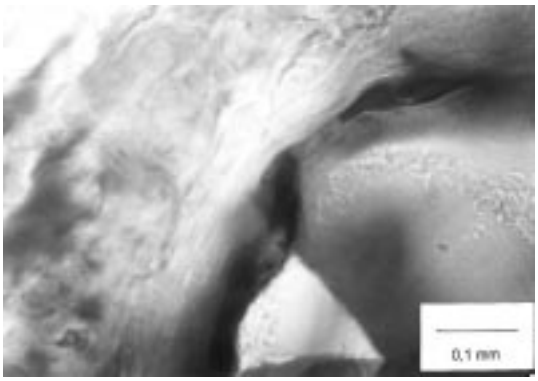

Figure 9 Response to bioactive degradable B01 glass elicits a very good response since bone remodeling proceeds even at the interface with bioactive glass. A tight apposition, without any fibrous interposition, is observed between bone and glass (transverse section, polarized light microscopy, 40 ×, at 8 weeks).

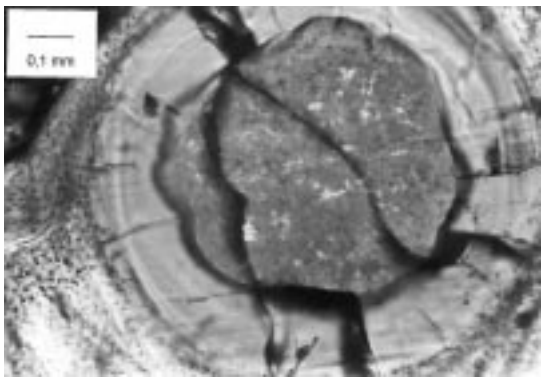

Figure 10 Similar to implants in muscle, the inner structure of bioactive degradable B01 glass implanted in bone is affected by the formation of an outer jelly-like cortex (transverse section, polarized light microscopy, 20 ×, at 8 weeks).

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