EVALUATION OF INTRAMUSCULAR TISSUE RESPONSE TO ZIRCONIA-HYDROXYAPATITE (ZH) CERAMIC IMPLANTS IN DOGS

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Abstract. *The purpose of this study is to evaluate the soft tissue response to two muscle zirconia-hydroxyapatite implants of different compositions in dogs by macroscopic and histological observations, and assess the characteristics of the new tissues formed on the surfaces of the implants as well as their biocompatibility under test conditions. The implants were inserted subcutaneously into the dorsal muscle of eighteen dogs, removed 30, 90 and 120 days after implantation, and finally examined macroscopically and by light microscopy. Although no forein body reaction or necrosis has been observed at the implantation sites investigated, the lack of macroporosity and the low degrees of microporosity and bioactivity inherent to these implants may have not provided suitable microstructure and microenvironment for cell differentation and bone matrix formation as a consequence of their non-inductive and non-conductive behaviors in soft tissues.. However, investigations concerning implantation at bony sites already in progress in order to assess the suitability of these candidate materials for future applications as high load-bearing implants.*

Keywords*: zirconia-hydroxyapatite composite, soft tissue, biocompatibility*

1. Introduction

The use of ceramic prostheses for the restoration and substitution of damaged human body parts has increased significantly during the last years (Kroon and Freeman, 1992; Daculsi, 1998; Qiu, Ducheyne and Ayyaswamy, 1999; Shinohara *et al*., 2000; Hallman *et al*., 2001). Biocompatible ceramics are composed mainly of calcium, sodium, potassium, silicon, phosphate, carbonate, and hydroxyl ions commonly found in the physiological environment, and of zirconium, aluminum and titanium oxides, which show none or limited toxicity to body tissue. Zirconia (Zr_2O), which is much tougher than alumina (Al_2O_3), is one of the newest and most promising bioceramics (Hulbet, 1993). A certain interest in zirconia applications for bone surgeries or as devices requiring good and reliable mechanical performance has spread in the biomedical field. In addition, zirconia is considered an inert non-bioactive ceramics, because when implanted, it only shows morphological fixation to surrounding tissues, without any chemical or biological bonding (Ferraris *et al*., 2000) In contrast, different calcium phosphate ceramics (CPC), mainly hydroxyapatite (HA), tricalcium phosphate (TCP), and tetracalcium phosphate (TTCP) present a particular interest as materials for biomedical grafts (Ravaglioli and Krajewiski, 1992; LeGeros, 1988), because they provide appropriate osteogenetic activity. However, these materials present very low mechanical strength and thus their biomedical application has been limited exclusively to areas free of dynamic load-bearing (Homes and Roser, 1997).

The assessment of *in vivo* compatibility of biomaterials and their efficacy as medical devices is a critical aspect for the development and use of implants in humans. While *in vitro* systems yield important fundamental information about certain elements of cellular and molecular interactions with biomaterials, they cannot replace *in vivo* evaluation. The

use of animal models to simulate human physiology is necessary to account for the biological processes comprising tissue response related to implant factors. This implies the utilization of standard practices which recommend sufficient biological testing to establish a reasonable level of confidence concerning tissue response to a given material or device to an adequate animal model.

Recent studies involving the synthesis, physical-chemical and microstructural characterization, determination of mechanical and biological behavior of calcia partially stabilized zirconia, hydroxyapatite (ZH) composites (Silva, Lameiras and Domingues, 2001; Silva and Lameiras, 2000) reported that these bioceramics possess great potential for future applications as high load-bearing implants. The addition of a particulate calcia partially stabilized zirconia phase to a hydroxyapatite one led to an improvement in the mechanical properties of these composites and did not affect their biocompatibility. Cytotoxicity assays results of cell culture, skin irritation, and acute toxicity by systemic and intracutaneous injection of powders, sintered bodies, and extract liquids of these material indicated they meet the standard practice requirements recommended in the evaluation of biological reactivity (Silva, Lameiras and Loato, 2002; ASTM, 1996).

The present work purposes to carry on these preliminary investigations from the estimate of tissue response of these composites to intramuscular implantation in dogs according to standard practices of biocompatibility assessment of candidate materials for short- and long-term implant applications (ASTM, 1987; ASTM, 1993).

2. Materials and Methods

2.1. ZH Ceramics

Two kinds of ZH implants, noted as Z4H6 and Z6H4 with 40 and 60 vol. % in zirconia phase, respectively, were previously prepared by a coprecipitation method (Silva, Lameiras and Domingues, 2001; Silva and Lameiras, 2000) followed by calcination, pressing into cylindrical shape, and posterior sintering (Silva, Lameiras and Domingues, 2001). The complementary phase was constituted by hydroxyapatite. The zirconia phase consisted of a calcia partially stabilized zirconia system. The average dimensions of the implant pieces were 5 mm in diameter and 2 mm in height.

2.2. Animals

Eighteen healthy adult male mongrel dogs weighing from 10 to 20kg were used as test hosts. They were randomly divided into three groups of six each, according to implantation time: Group 1: 30 days; Group 2: 90 days, and Group 3: 120 days. The animals were assigned as follows: animals 1 through 6 to Group 3, 7 through 13 to Group 2, and 14 through 18 to Group 1. They were housed in standard conditions and supplied with food and water *ad libitum.*

2.3. Surgical Technique

Surgical proceedings were conducted on all test animals under preanaesthesia and sedation with atropine solution (0.044 mg/kg) and xylazine solution (1.0 mg/kg), respectively, and sterile conditions. An intravenous preoperative injection of sodium cefazolin (30mg/kg) was also applied to each preanaesthetized dog thirty minutes before surgery. Back hair was shaved to expose two test areas on each side of the back. Fifteen minutes later, the animals were anaesthetized by intravenous injection of sodium thiopental solution (12.5mg/kg) and maintained with halothane (2%) and oxygen (100%). The animals were placed in sternal recumbency and a longitudinal skin incision (3.0cm in length) was made by scalpel at both the center and the middle of the back, and *longissimus dorsi* muscle bundles on both sides were disclosed. Odd number-labeled animals had Z4H6 pieces implanted into the right side and Z6H4 pieces into the left side of the back. Implant side positions were switched for even number labeled animals, that is, Z4H6 was inserted into the left side and Z6H4 into the right side. All incisions were thoroughly irrigated with saline solution closed in layers.

2.4. Histological Preparation

The animals were pharmacologically euthanized under general anesthesia by an overdose of sodium thiopental 2.5%, and the implants were collected with surrounding tissues for morphological investigations. Implants were removed before submitting soft tissue samples to histological preparation.

Samples were fixed in 10% buffered formaldehyde, then dehydrated in a graded series of alcohol solutions from 70% to 100%, and embedded into paraffin. Cross-sections of 5μm were made and stained with hematoxylin and eosin (HE).

3. Results

3.1. Macroscopic Observation

The animals appeared to be in good health throughout the experiment. No negative tissue response, such as inflammation, characterized by either hyperemia, or hemorrhage or swelling was found surrounding implant region. No adverse tissue reaction such as necrosis was observed for any of the implanted samples. There was no evidence of calcification surrounding the intramuscularly implanted samples as shown in Fig. 1 for the both implants (Z4H6 and Z6H4) after 30 days of implantation.

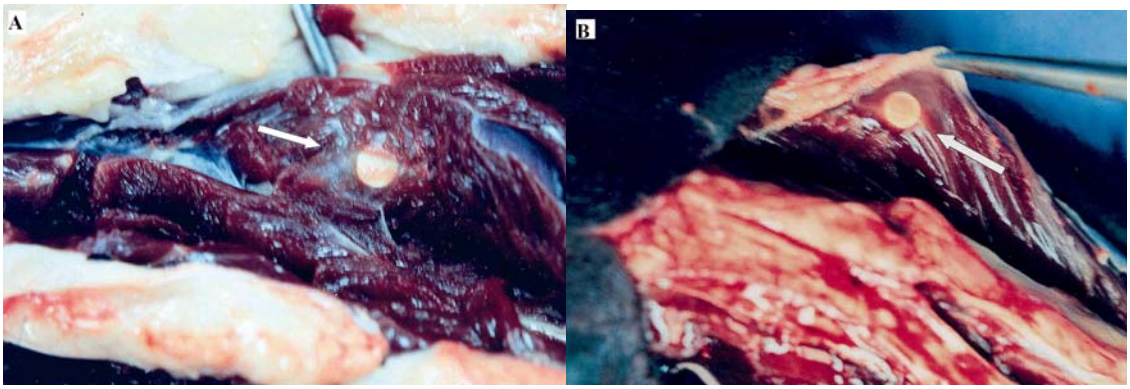

Figure 1 – Tissue responses after 30 days of implantation to (a) Z4H6 and (b) Z6H4 implants.

3.2. Light Macroscopic Observation

Intramuscularly implanted samples of neither Z4H6 nor Z6H4 composites exhibited bone formation in histological evaluation. After the first thirty days after implantation, both implants were covered with a thick layer of fibrous connective tissue, which was remodeled into a denser type and fibers were arranged mainly parallel to the surface (Fig. 2a and 2b). The fibrous capsules were sandwiched between the normal surrounding muscular tissue and the implants. Inflammatory response was characterized by high count of cells such as macrophages and lymphocytes attached onto the implant surfaces. Small implant particles were also found detached from the main implants (Fig. 3a and 3b).

Ninety days after implantation, small areas of multinucleated giant cells could be observed in direct apposition to the implant. Proliferation of connective tissue surrounding implants was observed, but it was lower than in the 30-day evaluation (Fig. 4a and 4b).

After a prolonged implantation period (120 days), the implants were covered with a thin layer of connective tissue. No inflammatory response was found (Fig. 5a and 5b).

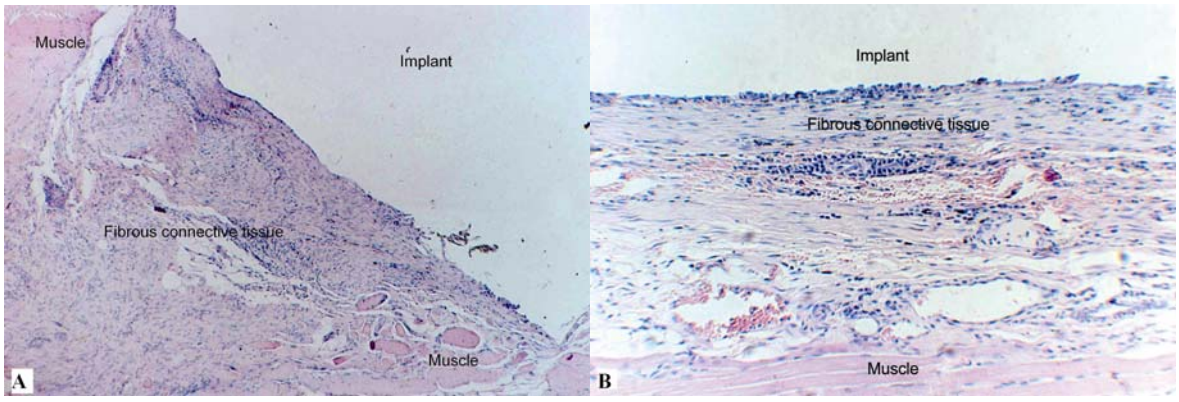

Figure 2 – Histological observation in the *longissimus dorsi* muscle of dogs after 30 days of implantation around (a) Z4H6 and (b) Z6H4 implants (original magnification X 40).

4. Discussion

Intramuscular implantation results indicate that the ZH implants investigated are biocompatible as neither foreign body reaction nor necrosis was observed surrounding the implanted samples (Hulbet, 1993; Ravaglioli and Krajewiski, 1992; Silva, Lameiras and Lobato, 2002). Otherwise, microscopic observations revealed a similar inflammatory response for both ZH implants (Fig. 3a and 3b), which is commonly found for implant materials. However, there was no induction of bone formation.

The important environmental factors which are probably involved in bone formation induction in non-bony sites are: (i) the interconnected macroporous structure of the implant as it facilitates ingrowth of blood vessels and cells; (ii) the microporous structure of the macropore walls as it increases the protein adsorption areas and may provide a favorable surface for cellular adhesion and differentiation; (iii) the biomolecules and crystallized apatite layers deposited onto the pore surface in the earlier stages; and (iv) locally increased calcium ion concentration resulting from the degradation and dissolution of the ceramics (Yamazaky and Sakai, 1992).

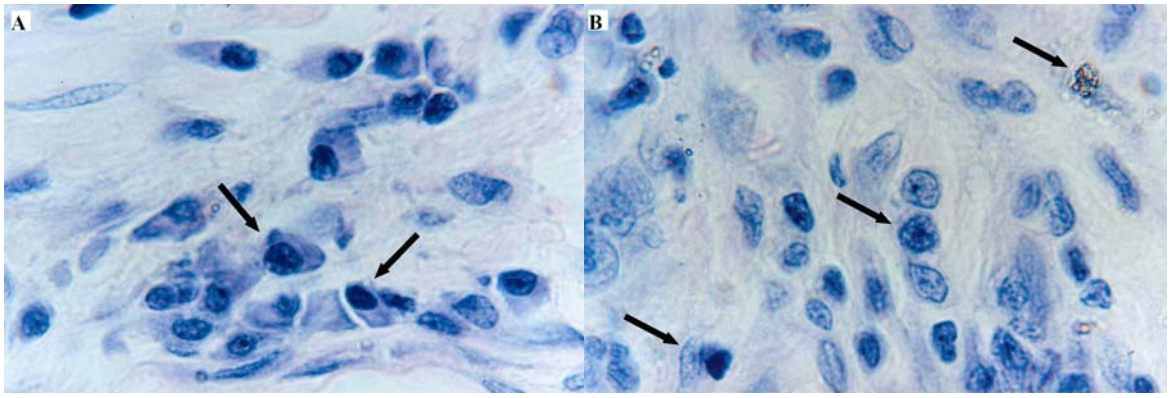

Figure 3 – Histological observation in the *longissimus dorsi* muscle of dogs after 30 days of implantation, showing macrophages and lymphocytes on the particles of the (a) Z4H6 and (b) Z6H4 implants (original magnification X 100).

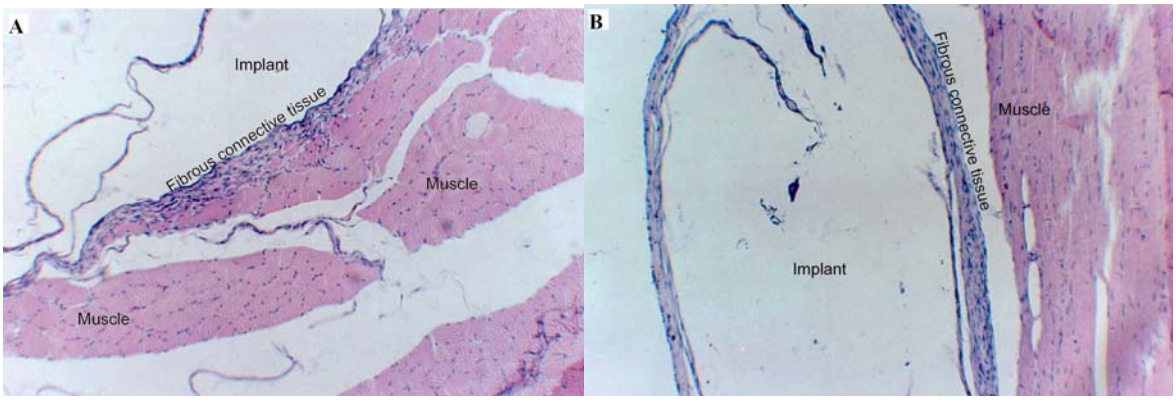

Figure 4 – Histological observation in the *longissimus dorsi* muscle of dogs after 90 days of implantation around (a) Z4H6 and (b) Z6H4 implants (original magnification X 40).

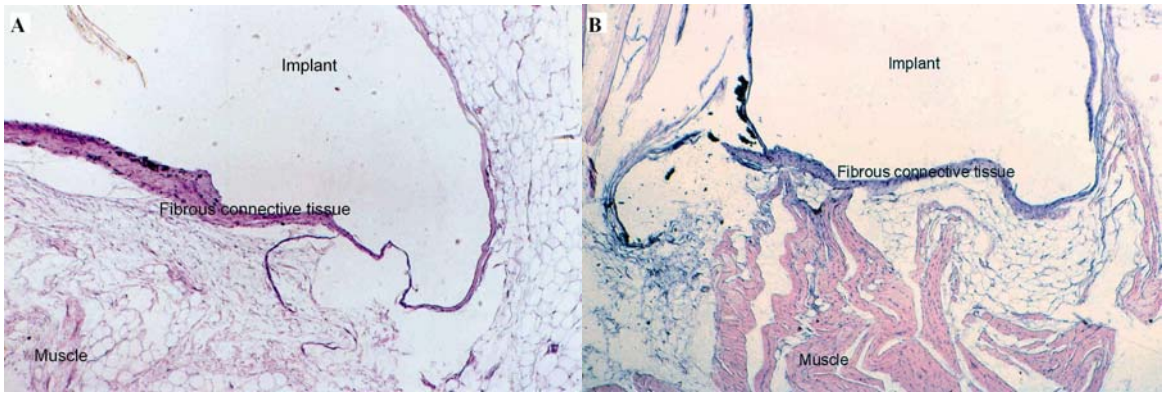

Figure 5 – Histological observation in the longissimus dorsi muscle of dogs after 120 days of implantation around (a) Z4H6 and (b) Z6H4 implants (original magnification X 40).

It is suggested that osteogenesis is induced by physical and chemical factors in the micromilieu provided by porous ceramics granules. One of the composite properties used in this work (Z4H6 and Z6H4) is densities of 85 and 90%, respectively (Silva, Lameiras and Domingues, 2001). Densification around 90% is desirable because the presence of a certain degree of porosity contributes to bone inter-growth and a subsequent implant-bone interfacial adhesion (Silva, Lameiras and Domingues, 2001). However, stoichiometrically dense hydroxyapatite limits *in vitro* and *in vivo* reactivity in soft tissues (Yuan *et al*., 1999), which is clearly demonstrated by the absence of bone formation at the site of implantation of the composites used at this work.

In a study evaluating soft tissue response to subcutaneous implantation of porous and dense HA ceramic granules, osteoblast differentiation and new bone formation at the surface of implanted porous HA ceramic granules were demonstrated. In contrast, there was no evidence of newly formed osseous tissue at the dense HA ceramic granule implantation sites (Yuan *et al*., 1999). Tissue formation in the porous region of the extraskeletally implanted ceramics followed a complex process involving blood clot formation, vascular invasion with the subsequent formation of granular tissue, polymorphic cell aggregation, osteoblast differentiation, and ensuing bone formation. It is suggested that osteoblastic differentiation occurred within the cell clusters aggregated on the pore inner surface, which secreted bone matrix when in direct contact with the ceramics (Yang *et al*., 1997).

Previous studies showed that the formation of a carbonated surface between implant and soft tissue is related to the material dissolution rate (Yuan *et al*., 1999). Slow dissolution stimulates proliferation, aggregation, and the differentiation of cells bone progenitors and the subsequent formation of bone matrix (Ducheyne and Qiu,1999; Passuti *et al*., 1989). The dissolution of calcium phosphate bioceramics depends on their compositions, Ca/P ratio, impurity, and structural features (Silva, 1998). In addition, the bioactivity index of the stoichiometric dense hydroxyapatite reveal that this bioceramic is not able to make an interfacial bonding enough strength to soft tissues, while zirconia ceramics develop a non-adherent fibrous capsule as implanted in either bony and non-bony sites, and are nearly bioinert (Hench and Kokubo, 1998). Ceramics with Ca/P molar ratio of 1.67 are stable and thus are said to be pure (Silva, 1998; LeGeros, 2002). Among all CPCs studied, stoichiometrically dense hydroxyapatite dissolved the slowest (Passuti *et al*., 1989). However, even though this material can react with surrounding bone tissue, its reaction rate is very low. As competing reactions take over quickly, bone tissue formation does not take place on HA surface (Passuti *et al*., 1989).

Recent reports showed the capability of some calcium-phosphate ceramics (CPC) for ectopic bone formation (osteoinduction or material-induced osteogenesis), which seems to be both material- (Yuan *et al*., 1999) and animal-dependent (Yang, 1996). A major advance in bone cell biology has been the recent characterization of a new family of gene-related products belonging to the bone morphogenetic protein (BMP) family. BMPs are molecular initiators that regulate cartilage and bone differentiation *in vivo*. Different concentrations of putative circulating or locally produced BMPs determined by different skeletal homeostasis in animals may be responsible for the morphological differences observed between species (Ripamont, 1996).

The lack of macroporosity, in addition to the low degree of microporosity and non-bioactive behavior in non-bony sites found in the Z4H6 and Z6H4 implants (Silva, Lameiras and Lobato, 2002), mainly associated to the hydroxyapatite and zirconia phases present in the composites, may not provide suitable microstructure and microenvironment for cell differentiation and bone matrix formation in soft tissue due to their non-inductive characteristics. Furthermore, the addition of calcia partially stabilized zirconia to the hydroxyapatite matrix might contribute to the lower osteoinduction capacity of these composites when implanted into soft tissue.

4. Conclusions

The intramuscular tissue response to two different composites of calcia partially stabilized zirconia-hydroxyapatite (Z4H6 and Z6H4) in dogs was investigated through macroscopic and histological observations 30, 90, and 120 days after implantation. Our data showed that these composites are biocompatible, which is an important property since they have been considered for medical use. Although these materials are biocompatible and mechanically resistant, the results suggest that they do not present inductive properties as a consequence of the lack of macroporosity, the low degree of microporosity and non-biactive behavior required for inducing bone formation in soft tissue. However, investigations concerning implantation into bony sites are already in progress in order to assess the suitability of these candidate materials for future applications as high load-bearing implants.

5. Acknowledgements

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