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Characterization of glass ionomers for surgical applications

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The clinical use of glass ionomers is well established in the odontological field. In particular; (i) high biocompatibility (unincreased macrophages activity), and consequently no inflammation in post-operative course; (ii) total non-toxicity of components; (iii) absence of mutagenic consequence on osteoblasts; (iv) dimensional stability and absence of macroscopic changes after very long implantation times; (v) stability to water and biological compounds, make these materials attractive for further clinical applications. The high adhesion both to metallic or ceramic surfaces and bone tissue makes this materials excellently usable for the adjustment of bone defects. This goal necessitates suitable knowledge of the mechanical, chemical, physical and biological properties of commercial materials so that the most suitable product can be identified. The characterization of representative products of each of the principal classes of glass ionomers material is reported.

1. Introduction

Glass ionomers (or more correctly "glass polyalkenoates"), introduced by Wilson and Kent in 1971 [1], were intended for use in conservative dentistry. The name ionomer is derived from ionic polymer, which is obtained from the salification of a polycarboxylic acid. They have been used in dental practice for several years for their physiologic setting temperature, efficient marginal seal and high biocompatibility, but the most important property of the materials is the permanent adherence to hydroxyapatite of enamel and dentine when applied in moist conditions [2, 3]. This unique property contributes to the favourable mechanical stability of implants. For many years attempts have been made to develop the ideal artificial material for the replacement of bone tissue. At present, a material which exactly meets all requirements is unavailable, but great improvements have been made recently using materials already utilized in the odontological field. At present, ionomeric bone cements have been used with success in different medical fields, e.g. in surgery as bone tissue replacement (subsequent to trauma, malformation or tumour).

Glass ionomers are synthetic composite materials with a discontinuous inorganic reinforcement (i.e. aluminum-silicated glass powder) dispersed in a cross linked polymeric matrix. They are obtained by reaction of inorganic powder (e.g. ion exchanger containing calcium, sodium, fluorine and phosphorus) with an aqueous solution of poly(acrylic acid) homopolymer or one of its copolymers. The glass component is usually a fluoroalumino–silicate, although glasses without fluoride have been used, either straightforward aluminosilicates or aluminoborates. The matrix is also reinforced by incorporating finely divided metal into the cement. The basic formulation can undergo a considerable change in the commercial products as in the new light-curing glass ionomers. The hardening reaction of "self-curing" (acid-base cements) is a salification of the polyacrylic acid by a basic glass [1, 2, 4–8]. The carboxylic groups of the polyacrylic acid are partially dissociated in aqueous solution and, therefore, an exchange with cations on the glass surface can follow. Decomposition of the glass under the influence of the polyacid, leading to release of Ca^{2+} ions and Al^{3+} species, these latter probably being in the form of complex oxyanions containing several aluminium atoms. A rapid reaction of the Ca^{2+} ions with the polyacid chains follows, and then a slower reaction of Al^{3+} species gradually released from the anionic complex. This exchange gives rise to the formation of a silica hydrogel layer on the glass particles and, in the matrix, the formation of intra and inter-molecular ionic bonds. The first electrostatic cross linking degree is low because only cations with a low electric charge density (i.e. calcium ions) are involved in the formation of saline bonds. The material, at this stage, is still hydrophilic and hydrosoluble and, therefore, it must be protected from moisture [9]. Subsequently, the calcium ions in the matrix are replaced by the aluminum ions which,

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being trivalent and with a high electric charge density, can assume a larger number of stronger electrostatic interactions. This gradual hydration of the silicate fragments leads to a matrix of increased strength and greater resistance to the drying process. The aluminum polyacrylate contained in the hardened material is insoluble in water.

A further development of this class of material has been the incorporation of photopolymerizable components to form hybrid cements known as light-cured glass–ionomers [7, 8, 10]. These became commercially available in the late 1980s. The advantage of these materials is that the initial set of the cement, the photochemical polymerization, gives the cement good early protection against attack by moisture. The early susceptibility of the simple acid-base glass-ionomers has long been recognized as a weakness and is overcome in clinical use by coating the cement with a varnish or petroleum jelly. Light-cured glass-ionomers consist of a complex mixture of components. These are: (i) poly(acrylic acid) or a modified poly(acrylic acid); (ii) a photocurable monomer such as hydroxyethyl methacrylate (HEMA), or a photocurable side chain grafted on to the poly(acrylic acid); (iii) an ion-leachable glass; and (iv) water. The initial setting reaction is the photochemical polymerization (a copolymerization of the HEMA with the polymer side-chains or a homopolymerization of the functional groups in the side-chains) and subsequently, the acid-base reactions typical of glass-ionomers takes place.

A further hardening process is the tri-cure mechanism that allows the extension of the reaction to locations far from the exposed surface. This second reaction provides a relatively rapid setting where UV-light does not penetrate. The system include two components (microcapsule of potassium and ascorbic acid), that, when react, produce a great amount of heat that completes the material curing [8, 10]. Although the broad outline of the setting reactions in these hybrid materials is clear, several points of detail remain to be elucidated.

This work analyses the possibility of application of glass-ionomer cements as bone tissue substitutes in the oral and maxillo-facial regions. Because of high mechanical stresses, the performances required of materials used in these regions are different from those in neuro-surgery and orthopaedic fields. An accurate caracterization of these materials is therefore an indispensable preliminary statement to the utilization of glass ionomers as bone tissue replacement in oro-maxillo facial prosthetic surgery. The identifications of more suitable materials is necessary. The characterization study was performed using differential scanning calorimetry (DSC), spectroscopic analysis and energy-dispersive X-ray analysis (EDS).

2. Experimental procedures

2.1. Materials

The cements used in this study are glass-ionomer systems commercially available, including self-curing (Ionocap 1.0 (ION), GC Fuji II (GCF), Ketac (KET)), photo-curing (LC Fuji II (LCF)) and tri-cure systems (3M Vitremer (VIT)). The premeasured encapsulated materials (Aplicap System) were automatically mixed by Ionomix-Mixer for 10 s at 4300 oscillations per minute; the other materials were manually mixed and homogenized. When the cement was a light-cured or a tri-cure glass ionomer the polimerization was performed using a Translux CL Kulzer lamp, producing a 470 nm wavelength UV-radiation.

2.2. Equipments

Particle size distributions of the ceramic powders were determined using a Microtrac Particle Size Analyser System, which employs a laser diffraction method. The powders were placed in distilled water and stirred continuously to aid dispersion of the fine particles.

The calorimetric analysis was performed by using a differential scanning calorimeter (Mettler DSC-30 apparatus), under dynamic nitrogen atmosphere (0.4 cm^3/min), with low temperature cell and TC11TA processor. The instrument was connected to a computer and equipped with a liquid nitrogen system for fast cooling. Isothermal tests were carried out at 37 °C. Dynamic runs were performed at a heating rate of 5 °C min^{-1} from 37 °C to 100 °C. Specimens of about 10–20 mg were encapsuled in standard high-purity aluminum sample pans. A similar aluminum sample pan was used as a reference.

With photopolymerizing materials the calorimetric cell covering was replaced by a refractory material disc, perforated for the introduction of the optical fibre of a UV-lamp.

Spectroscopic analysis was performed by a Nicolet 5PC FT-IR spectrometer. Infrared spectra were obtained ranging from 600 to 4800 cm^{-1}, with a resolution of 4 cm^{-1}. Very thin layers of the samples were put on a NaCl plate and fixed under an IR-laser ray. Isothermal tests were performed at 37 °C. The NaCl plate with the sample was placed in a stove for different times and then in a desiccator for 10 min.

Microstructural observations were carried out using a HITACHI S-2300 scanning electron microscope (SEM). The examination was performed at 20–25 kV and the specimens were coated with gold to reduce charging and to improve image quality.

The quantitative elemental analysis of the interface was performed by diffractometric methods using energy-dispersive X-ray analysis (EDS, in a SEM Cambrige Stereoscan), with Al and Si standards. A Bohlin Rheometer, with a torque element of 1.59 g cm, was used for the viscometer tests.

Mechanical properties of these materials were obtained using, for the compression tests an Instron machine mod. 4204, with a static load cell of 5 kN and for the microardness tests a microdurometer LEITZ Wetzlar with micrometrical eyepiece connected to a digital reader for impression evaluation. The test samples were made by hardening of cement in a heater, at 37 °C, using aluminum moulds. The resulting samples were cylinders of 5 mm diameter and 6 mm length. With photopolymerizing materials, transparent moulds were used. The samples for

hardness evaluation were hardened in a heater at 37 °C, moulded in DAP and then polished. The tests were performed using a load of 100 g (980.0 mN).

3. Results and discussion

3.1. Spectroscopic and thermokinetic characterization

The particle size distribution of the starting powders was obtained by SEM examination. The distributions were similar for all five commercial powders. The results showed that the powders consist of a bi- and tri-modal particle size distribution (see Fig. 1), presumably produced to enhance the packing density and thus minimize shrinkage. SEM observations of ceramic powders showed the irregular nature of the particles, the coarse powder morphology, and the well-facetted large size particles. The mean (26 μm) and the maximum particles size (53 μm) are similar for all the ceramic powders. Back-scattered micrographs of the microstructures of GCF, VIT suggests that there is phase separation in the glass matrix, which was not evident in the other materials (e.g. ION, Fig. 2). The microstructure consisted of individual crystals randomly and non-uniformly distributed throughout the glassy matrix.

Microcracking in the glass matrix, either around individual crystals or around crystals clusters was clearly evident. There were also, in some instances, cracks running from the glassy matrix into and through the crystals. The presence of microcracks around the clusters of crystals suggests that non-uniform shrinkage of the glassy matrix and crystalline phases had occurred on setting.

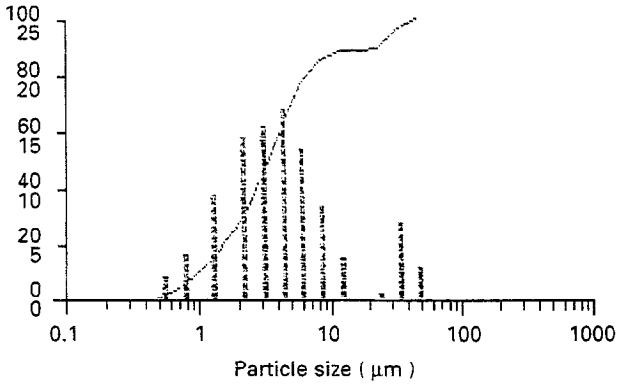

Figure 1 Particle size distribution of as-received ceramic powders for ION.

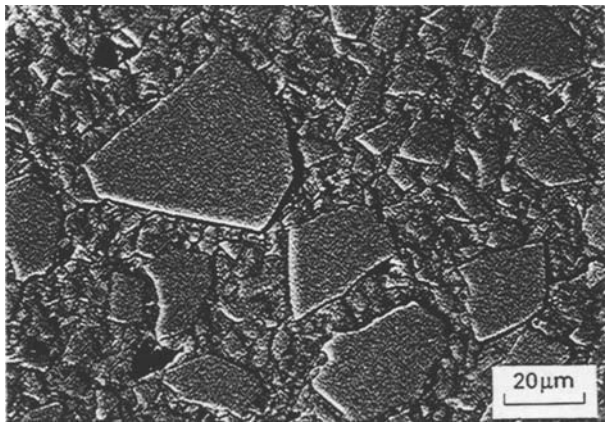

Figure 2 BSI micrograph of the ION surface. The phase separation is absent in the material.

A structural characterization of the glass ionomer cements and liquid precursors was easily obtained by infrared spectroscopy (FT-IR).

The thin and intense absorption band at 1715 cm^{-1} was distinctive of the carboxylic carbonyls of polyacrilic acid, and was produced by their stretching movement. The salification of these groups produced a shift to lower wave number (i.e. 1630 cm^{-1}). Nevertheless, the carboxylic resonance did not disappear at the end of the matrix cross linkage, showing that only a small carboxylic portion was neutralized in the hardened cement. The two absorptions were unresolved in the infrared spectrum. The strong and large absorption band at 3400 cm^{-1} was produced by hydroxylic groups stretching of solvent and by carboxyls. Water also produced the 1981 cm^{-1} band. The resonance at 2900 cm^{-1} was produced by the stretching movement of the carbon–carbon σ bonds that constituted the carbon framework of polyacrylic acid.

Therefore the basic constituent of hardened matrix was a saturated hydrocarbonical structure functionalized by carboxylic and carboxylate groups produced by polyacrilic acid or by its copolymers with maleic or tartaric acid. Further absorptions were visible in the photopolymerizable products denoting the presence of olefinic unsaturation.

The intensity of the hydroxylic absorption was significatively reduced during the hardening reaction. This indicated that the starting dimensional stability of the material was produced by water absorption from the hygroscopic inorganic powder.

The ionic exchange that characterizes the hardening reaction of cement is an exothermic process; the generated heat changed with chemical composition. A primary requirement in surgical applications is that the reaction heat does not overheat the organic tissue, that otherwise necroses.

The necessity to dispose of a chemical reaction having a not excessively high reaction rate is an additional requirement that allows a surgeon to prepare a well-homogenized material and to apply it in the best way to the bone. Materials that predictably best satisfy this requisite are the photo-curing glass ionomers, in fact (as verified with LCF and VIT) this special class of glass ionomers allows one to work the powder–liquid matrix mixture because the hardening process starts only when the material is irradiated with ultraviolet rays.

Reaction time (assuming it to be conventionally, the time after which thermal effects calorimetrically valuable are not present), reaction heat (opposite of enthalpy variation) and maximum thermal flow (Figs 3, 4 and 5) were determined for all the sample materials.

The detectable thermal effects finished with the hardening reaction and the following consolidation, i.e. Ca–Al exchange did not produce calorimetrically valuable heat.

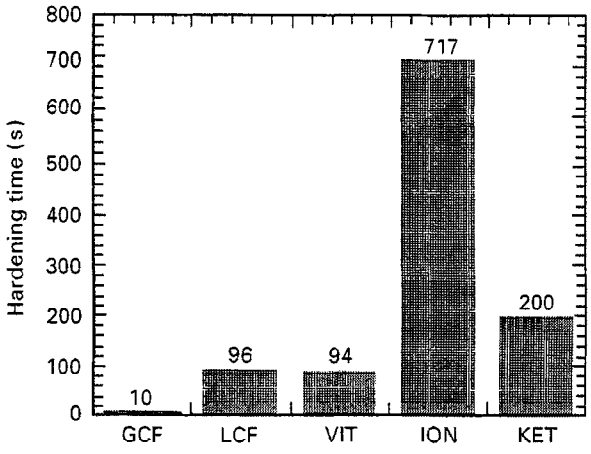

Figure 3 Histogram of the hardening time for the examined cements

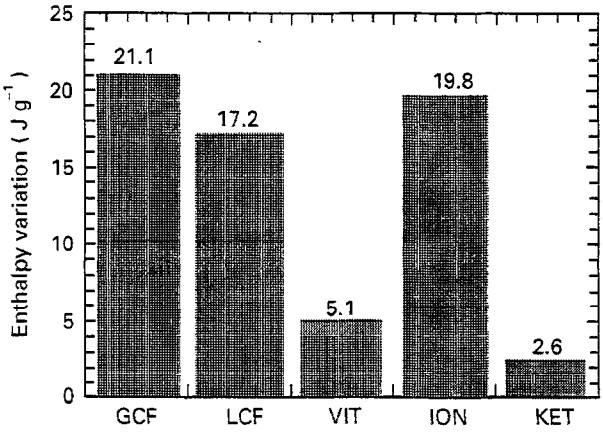

Figure 4 Histogram of enthalpy variation for the examined cements

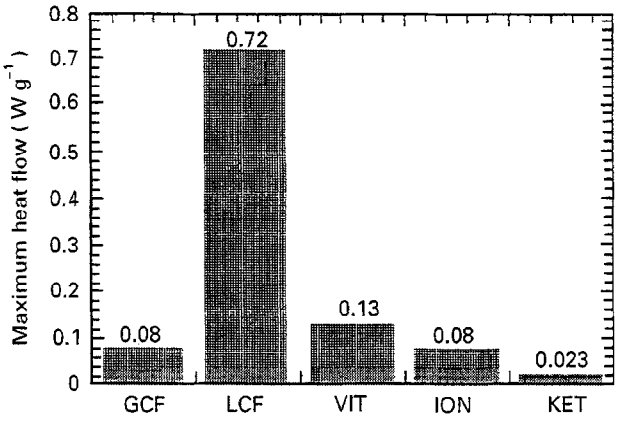

Figure 5 Maximum heat flow produced during the hardening reaction

The thermogram of the ION hardening reaction showed a behaviour characteristic of two concomitant exothermic reactions, whereas the other materials analysed showed a single exothermic reaction.

In addition, to establish the possible existence of residual reactivity, i.e. a post-hardening reaction (slow at physiological temperatures up to 37 °C) perfectly hardened samples were subjected to dynamic temperature range 37–100 °C with a heating rate of 5 °C min^{-1}. All analysed materials showed no thermal effects produced by post-hardening reaction, and the only process observed was cement degradation by water volatilization, which started at 65–70 °C.

3.2. Morphology of fracture surface

The microscopical investigation at low magnification of the fracture surface of tested glass ionomers showed the presence of a high level of spherical pores (see Fig. 6). These are due to shrinkage produced during cement hardening. In hardening, the material undergoes a remarkable volume variation, due to the absorption of matrix water by the glass particles. When the material assume dimensional stability, further contraction gave rise to the shrinkages. ION was the only material scarcely porous and was therefore more dense and compact (as determined by the microardness tests).

The examination at high magnification (1000–1500 ×) of the same area showed a surface distinctive of a brittle fracture of the material (Fig. 7). A little plasticization of the fractured matrix was visible only in photopolimerizable cements and particularly with the LCF product.

3.3. Mechanical characterization

Hardness is important information for the mechanical behaviour of material in service. Microardness tests

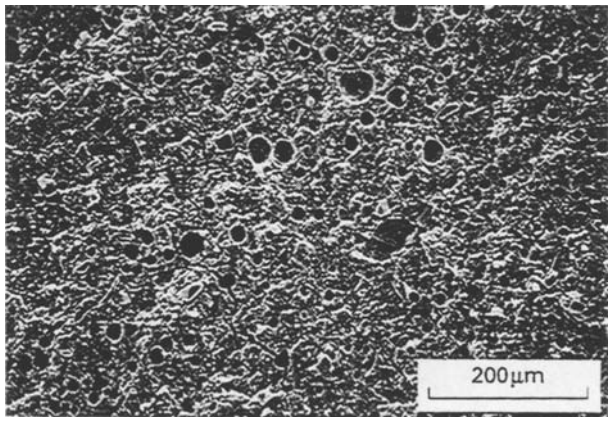

Figure 6 SEM micrograph of the fracture surface of the Vitremer glass ionomers

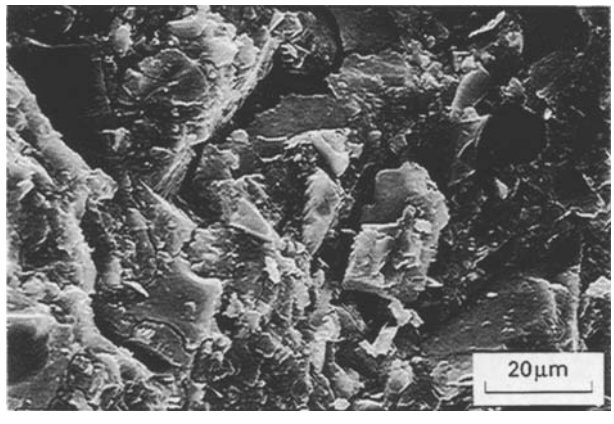

Figure 7 SEM photograph of the fracture surface of the Ionocap 1.0 glass ionomers

were performed on all the analysed samples and the values were compared with that of a cortical bone (see Fig. 8). The values were the average of six different determinations. The hardness of glass ionomer cements is similar to that of bone (very hard material) and of typical non-ferrous metals (e.g. titanium). Therefore, glass ionomers can constitute an excellent implantation cement.

The mechanical properties of glass ionomers were obtained through compression tests, because only a small quantity of material is required and the test reflects the more common stress in service. All the materials showed an elastic behaviour up to the break point. The compression test results (shown in Fig. 9 and Table I) indicated that the Young modulus of all tested cements was very different from that of bone. When a photoinduced reaction was present in the cement, the elastic modulus and the maximum supportable load were considerably increased.

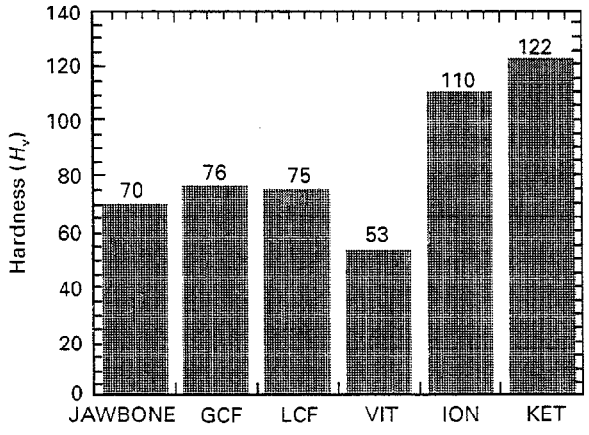

Figure 8 Comparison between the hardness values of bone and glass ionomer cements

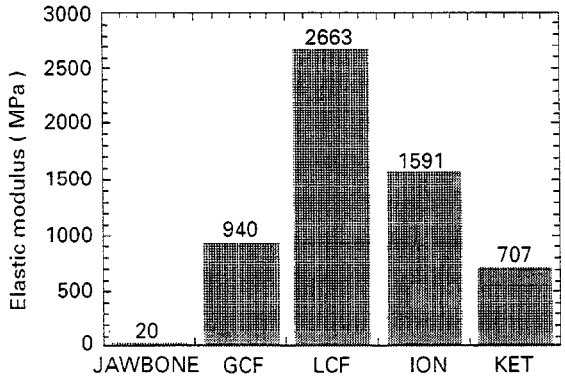

Figure 9 Comparison between the elastic modulus of the bone and those of the tested materials

TABLE I Results compression tests

Ionomer	E (MPa)	ε (mm/mm)	σ (MPa)
Vitremer	2143	0.1306	107.2
Ketac	707.5	0.1452	33.00
Fuji II LC	4165	0.0603	150.4
Fuji II GC	940	0.175	38.70
Ionocap	1591	0.0085	11.77

The transparence to UV-radiation was also analysed, observing that layers of photopolimerizable glass ionomers thicker than 4 mm were impenetrable to ultraviolet radiation. Even a very long exposure time (up to 40 s) under a concentrated beam (such as the light produced by a translux CL Kulzer ultraviolet lamp) did not allow total hardening of the material. Consequently, application is necessary for each subsequent layer and the resulting properties are lower than those of a single cement block. As a result, these light-cured cements appear recommendable in clinical use only as lining materials.

3.4. Adhesion mechanisms

The mechanism of glass ionomers–bone adhesion can be understood by microscopical investigation of the interface microstructure. The sample was prepared by spreading fresh cement on the exterior surface of a dry jawbone (sub-periosteal surface). The hardened material was incorporated in a synthetic resin, cut, ground and, after metallizing, examined by means of a scanning electron microscope (SEM) and energy-dispersive X-ray analysis (EDS). The microscopical investigation of the interface, conducted at high magnification (see Fig. 10) showed that during cement hardening, the fluid matrix segregated to the interface bone–glass ionomers, permeating the largest bone pores, and penetrated considerably into the bone (up to 40 μm, at some points). This for the low viscosity of the aqueous matrix (Table II), and the hight bone hydrophilicity. When the material hardened, the intrusion of cement into the bone assured a large contact surface area of bone–glass ionomers. The extension of contact between bone and implantation material is unsuitable

Figure 10 SEM micrograph of the bone-Ionocap 1.0 glass ionomer interface

TABLE II Visocisities values of some glass ionomer matrixes

Glass ionomer	Viscosity (Pa s)
Vitremer	0.0843
Fuji II LC	0.239
Fuji II GC	0.343

with scarcely biocompatible materials (e.g. the formation of an encapsulating layer of fibrous tissue observed near metal implantations), but here the high biocompatibility of the material renders this factor non-critical and is advantageous for adhesion. In addition, the hardened matrix contained in the bone pores gives rise to a very flexible connection. This mechanical anchorage play an essential role in the definition of adesion characteristic properties.

The hardening of the matrix penetrated into the bone was measured by X-ray analysis. The behaviour of the aluminum and silicon concentration normal to the interface was used as an index of the cross-linking degree. The aluminum concentration gradually reduced, permeating through the bone (Fig. 11).

As suggested from the spectroscopic analysis of the precursor and of the hardened glass ionomer the carboxylic group number was so high in the polyacrylic acid that only a reduced fraction of them remained, being mostly consumed at the end of the hardening reaction. A suitable adhesion between a synthetic polymer and a metal surface is possible only when the structural unit of the polymer contains acid groups that can react with the basic oxide layer (TiO_2) coating the metal surface. The existence of a remarkable quantity of unreacted carboxylic groups allowed the glass ionomers to adhere to the metal surface; the adhesion mechanism was promoted by the water present in the cement. The adhesion was measured by compression tests on threaded titanium pins cemented in glass ionomer (ION). The test result was given as the mass (kg) that produced yielding. A sample prepared as in Fig. 12 failed under a load of about 175 kg.

The titanium surface after extraction showed it to be coated with cement residues indicating a system collapsing by breaking of material contained in the pin thread. Consequently, in the case of threaded titanium pins (but probably also with nails because of the high friction produced by the rough surfaces of vapour-deposited titanium), yielding is determined more by the intrinsic properties of the glass ionomer cement than by the adhesion of cement to the metal.

3.5. Biocompatibility

In some medical fields, such as orthopedics and neurosurgery, on the basis of experiments carried out on animals [11–16], the possibility of using GIC in therapy has been tested. However, the investigations in the oro-maxillo-facial area are scarce.

Biocompatibility, i.e. the ability to avoid adverse body reaction, is a distinctive property of biomaterials. Biocompatibility does not imply that the material is inert, but it must evoke a dynamic response that leads to a good result. So the general interest is directed either to bioactive materials, able to give linkages with bone and an osteogenetic activity, or to biodegradable materials if only temporary presence of the material is needed.

Therefore, we have tested *in vitro* the biocompatibility and bioactivity of all the GIC already mentioned. For each material we have to consider two principal aspects, biological and mechanical compatibility to accomplish its function over time.

The evaluation of the mechanical compatibility of a prosthetic material is carried out by experiments on animals; these *in vivo* models are also essential for human use. Biological compatibility can be tested using, first, *in vitro* models, and then experiments on animals for testing materials found to be citocompatible.

On this basis and in order to evaluate *in vitro* the biocompatibility, osteintegration and osteinductive ability of prosthetic materials, we have developed as a testing model, primary cultures of human osteoblasts from cancellous bone (thigh bone, shin bone, maxilla, mandible).

With this test model we have carried out a biocompatibility study which is one of the few existing that use human bone cells.

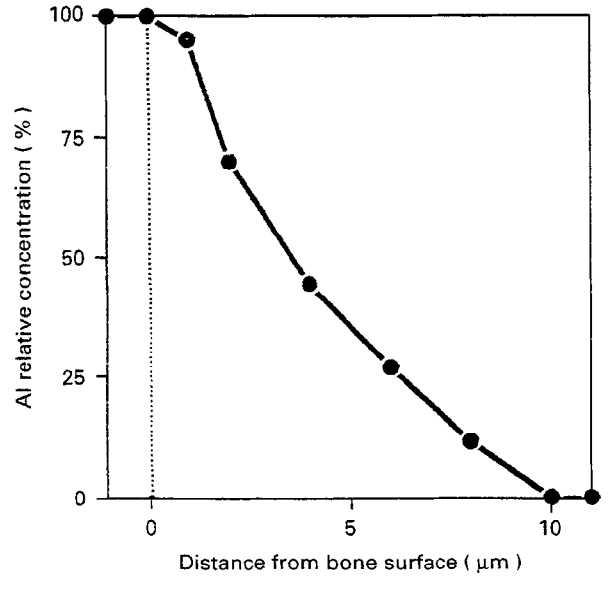

Figure 11 Experimental results on the Al concentration in the bone by microprobe analysis

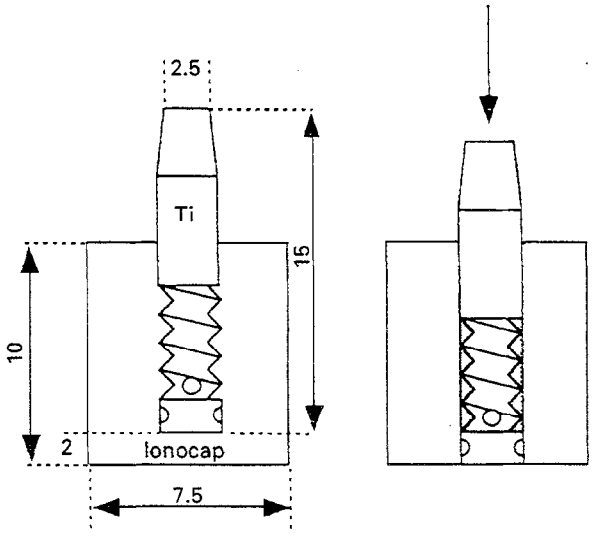

Figure 12 Mechanism of material yielding during the compression test performed on test sample titanium–glass ionomer cement (dimensions are in mm).

We used two successful methods to isolate cells [7, 8, 10, 17]. The first is the release of cells after bone sliver digestion by collagenase; the second is the cells migrating ability away from bone explant. The growing field is DMEM (Minimal Essential Medium modified by Dulbecco), which contains 10% FBS (Fetal Bovine Serum), antibiotics and ascorbic acid. Each GIC was prepared, following the manufacturer's instructions, in a sterile environment under a laminar flow cloak. Each GIC was stratified in order to cover the bottom of Petri plates of 2 cm diameter (multiwell of 6), with a layer of 1 mm of thickness. The osteoblasts were characterized on the basis of the morphological and biochemical properties and their answers to vary hormones and growth factors [7, 8, 10, 17]. Proliferation and adhesiveness to the material were also evaluated.

The EM examination pointed out the typical ultrastructural aspect of cells in active protein synthesis.

Inside the cells there were rough-surfaced endoplasmic reticulum, well-developed Golgi apparatus, mitochondria and several deposit vesicles. Outside the cells, collagenous fibres that are in close touch with the cell body are present.

One of the biochemical parameter used in order to identify the osteoblastic phenotype is the high activity of alkaline phosphatase and its increase after stimulation by 1.25–dihydroxycolecalciferole.

The kind of synthetized collagene was another important parameter. By using specific antibodies we found only type I collagene (not type III), confirmation of osteoblastic phenotype maintenance and of fibroblastic contamination absence.

The production of a specific protein, osteocalcine, after stimulation by 1.25-dihydroxycolecalciferole was definitive confirmation of the cells' osteoblastic feature. We underline that the gene of such protein is expressed only in the osteoblastic cells and that the transcription is directly dependent on the presence of the active hormonal form Vitamine D3.

To verify sterility and pH steady-state, the culture medium was added to plates containing the materials. In all cases the sterility was guaranteed. With regard to pH, a different response was observed for each material. More precisely, KET did not modify the DMEM pH, while the other GICs produced a decrease (between 5 and 6). Therefore for such materials before reaching the physological pH steady-state, several changes of medium were needed. In particular, VIT needed 12 changes, i.e. 60 ml of DMEM.

$1.5–2 \times 10^5$ osteoblasts were added to the plates containing the samples. Two different cell preparations were used: the first from the femur of a 70-year-old woman having undergone hip prosthesis surgery; the second from the scapular esostosis in a 4-year-old child.

The cells were observed directly by microscope in the plates whose bottom was partially covered by the biomaterial, because the observation using completely covered plates was not possible.

In all cases morphologically normal osteoblasts could be seen on the bottom, also touching the biomaterials. Only in the plate containing VIT, were the cells in suspension and unable to touch the bottom. With regard to KET, from the beginning of the experiment we noted a greater ability of the cell for adhesion and proliferation. Therefore, after cells confluence (about 1 week), to verify the osteoblastic phenotype the cells were incubated for 48 h with 100 nM of 1.25–dihydroxycolecalciferole. In the control plate and in that containing the tested material, comparable amounts of osteocalcine were found. This result showed clearly that the interaction between KET and the cells did not modify the expression of the osteoblasts specific marker.

$1.5–2 \times 10^5$ osteoblasts were placed in a plate and its bottom was completely covered with the material. After 24 h the medium was withdrawn and the contained cells were counted. In all samples, except for VIT, cell numbers were 5–10% of the starting number. We suppose that the remaining amount adhered to the examined biomaterial. In all samples, after removal to a new multiwell, we could see that an expressive percentage of cells not adhered to the biomaterial was still able to fasten to the new plate, showing that the cells were vital. In contrast, with VIT the cells were not totally linked to the material, but remained in suspension in the medium. Moreover, when the medium was again put in the plate, cells were not able to adhere to the new plate. This result showed that the examined material was cytotoxic.

4. Conclusions

From data obtained, it is possible to draw the following conclusions:

(1) a brittle fracture characterizes all the materials;
(2) shrinkage is present in the cements;
(3) the material hardness is comparable with that of bone and non-ferrous metal implantations;
(4) the modulus of elasticity of glass ionomers is very different from that of bone;
(5) a strong and elastic adherence characterizes the contact with bone;
(6) the exothermic reaction of hardening does not gives rise to excessive physiological tissues heating;
(7) some glass ionomer cements are non-toxic in bulk. Instead, normal haemopoetic and osteoblastic activity takes place on the cement surface, and this explains the total absence of fibrous tissue envelopment.

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