Coating titanium implants with bioglass and with hydroxyapatite

A comparative study in sheep

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Summary. *This study compares the osteointegration of titanium implants coated with bioglass (Biovetro GSB formula) and with hydroxyapatite (HAP). Twenty-four bioglass-coated and 24 HAP-coated cylinders were implanted in the femoral diaphyses of sheep, and examined after 2, 4, 6, 8, 12, and 16 weeks. The HAP coating gave a stronger and earlier fixation to the bone than did bioglass. Bioglass formed a tissue interface which showed a macrophage reaction with little new bone formation activity. In contrast, HAP, showed intense new bone formation, with highly mineralised osseous trabeculae in the neighbourhood of the interface.*

Résumé. *Le but de cette étude expérimentale est de comparer l'ostéointégration d'implants en titane recouverts de bioverre (Biovetro GSD) et ceux recouverts d'hydroxyapatite (HAP). 24 cylindres recouverts de bioverre et 24 recouverts d'hydroxyapatite ont été implantés dans la diaphyse fémoral du mouton, puis examinés à 2, 4, 6, 8, 12 et 16 semaines de l'implantation. Les implants recouverts d'hydroxyapatite montrent une fixation à l'os plus précoce et plus solide que ceux recouverts de bioverre. Le bioverre a été à l'origine d'un tissu d'interface os-implant avec présence d'une réaction macrophagique avec petite activité de formation d'os nouveau. L'hydroxyapatite en revanche, est à l'origine d'une formation assez intense d'os nouveau avec des trabécules bien minéralisés au voisinage de l'interface os-implant.*

Introduction

Bioactivity is the characteristic of an implant which allows it to form a linkage with the living tissues. Materials which are not bioactive tend to form a layer of fibrous tissue at the interface between implant and bone. The main difference between HAP (Hydroxyapatite) and bioglass is that the bioactivity of glass can be controlled by modifying its chemical composition. Traditional glass contains at least 65% silicon oxide, which gives it great resistance to humidity, but leaves it biologically inactive. However, Hench [7] demonstrated the bioactive properties of bioglass when it contained 40–45% of silicon oxide, 20–25% of sodium oxide, and 20–25% of calcium oxide.

GSB (Grupo Studio Biovetro) and Stazione Sperimentale del Vetro di Murano [1, 3, 4, 11] developed the only prosthesis in clinical use which is coated with Biovetro (CRM bioimplant). Its chemical composition is different from bioglass of Hench. The chemical composition of Biovetro is: (Na₂O: 7–24%, K₂O: 2–8%, CaO: 9–20%, MgO: 0.1–2%, Al₂O₃: 0.1–2%, SiO₂: 46–63%, P₂O₅: 4–8%).

To sum up, we feel that both coating biomaterials (HAP and bioglass) are a step forward in research aimed at attaining the ideal biological integration of an inert implant (hip replacement) into a living receptor bone which must bear considerable loads and permit extensive movement [6, 12, 13].

The aim of the study was to compare the degree of integration into the bone structure of titanium cylinders coated with HAP with those coated with bioglass.

Material and methods

The surgical procedures were performed in accordance with the standards established by the European Community for experimentation with animals [9].

A total of 54 titanium alloy cylinders were implanted (Orbimed). Each cylinder measured 3 mm in diameter by 10 mm in length. They were smooth-surfaced implants. Twenty-seven were coated with Biovetro and 27 with HAP using the Plasma-Spray system, which yielded a coating thickness of 80 μm . The cylinders had a socket at one end with a screw thread so as to permit the attachment of a device to allow removal. All were sterilised with ethylene oxide.

The cylinders were implanted in the distal femoral epiphysis in six Spanish Churra sheep, five years old, average weight 45 kg. The sheep were sedated by the administration of intramuscular xylazine at a dosage of 8 mg per kg of body weight and of intravenous ketamine (5 mg per kg). This allowed a sedation time of 15 to 20 min.

Using X-ray guidance the distal portion of the femoral diaphysis was located. A 1 cm cutaneous incision was made and through this an 8 mm perforation was made into the bone cortex using a cannular device. The trochar was immediately withdrawn, the implant introduced through the cannula, and then hammered home.

The same procedure was repeated for each cylinder, retaining a separation of at least 3 cm in each case and moving along towards the proximal end of the femoral shaft. The exact positioning of each cylinder with respect to the diaphysis was checked radiographically. When the right femur, where the cylinders coated in bioglass were implanted, had received its quota, the sheep was turned over, and the same operation was performed on the left side, where the four HAP-coated cylinders were inserted.

The animals were slaughtered after 2, 4, 6, 8, 12, and 16 weeks by intravenous injection of 40 milliequivalents of potassium chloride. Both femura were extracted by removing the soft tissues around them and were immediately split into four blocks, each containing a cylinder. The blocks were kept in 100% alcohol to prevent biological degradation.

Observations undertaken

In order to establish the differences between the two types of coating, a JEOL-JSM-T330A model scanning electron micro-

scopic (SEM) with a resolution of 4.5 nm, and 15,000 to 20,000 magnification capacity was used. The surface of each type of coating was examined at magnifications of 200, 350, 750, and 1,500.

A comparative study of the porosity of the surface of both coatings was carried out by means of a mercury intrusion technique. For this, a mercury porosimeter of the Micromeritics Autoporo II 9215 model was used, having two compartments for low pressure and a high pressure chamber with stainless steel walls.

Immediately after the operation radiographs were taken and these were repeated each month in order to check the state and position of the cylinders and any possible reaction of the surrounding bone. Any changes in the femoral structure were checked, together with all periosteal and endosteal reactions for both types of implant, with any differences in radiographic density being carefully observed in each case.

All cylinders were subjected to a mechanical extraction test until removal or failure of the interface using a universal servo-hydraulic machine (Instron, model 8501). The bone and cylinder blocks were placed onto the metal platform of the machine, to which they were secured by an appropriate assembly. The speed of movement was set at 1 mm/min in accordance with other transcortical models.

Observations were made of the biomechanical values which best represent the characteristics of the implant-bone interface. These are the peak load, namely the force needed to loosen the cylinder, and the shearing strength, which is the load per unit surface area of contact relating to the peak load. Only the cortical bone is considered relevant in calculating the surface in contact with the bone, since the cancellous bone of sheep is virtually all fat.

Once the cylinders had been extracted from the blocks of bone the latter were examined histologically. The pieces of bone were processed without decalcification and embedded in polymethylmethacrylate. Glass containers were filled with a methyl-methacrylate mixture, the blocks were placed in these, hermetically sealed and kept in an oven at 32°C until hardened.

Using a microtome, sections were taken perpendicular to the axis of the femoral shaft from the bone blocks from which

Table 1. Mechanical test, bioglass results

Time (weeks)	Sample	Cortical bone width (mm)	Peak load in newtons (N)	Shear strength in megapascals (Mpa)
2	1	2.65	22	0.88
2	2	2.7	25.4	1.00
2	3	2.3	61.7	2.85
2	4	2.8	98.8	3.74
4	1	3.2	151.8	5.03
4	2	3.1	364.7 ^a	—
4	3	3.3	80.9	2.60
4	4	2.9	34.0	1.24
6	1	2.65	36.2	1.45
6	2	2.6	151.9	6.20
6	3	2.9	41.9	1.53
6	4	2.65	74.6	2.99
8	1	2.1	104.8	5.3
8	2	2.7	238.6	9.38
8	3	3.5	413.0	12.52
8	4	—	29.9 ^a	—
12	1	2.97	321.3	11.48
12	2	2.97	94.0	3.36
12	3	2.97	320.5	11.45
12	4	2.97	184.3	6.58
16	1	3.8	270.7	7.56
16	2	3.6	190.4	5.61
16	3	4.0	187.4	4.97
16	4	3.1	608.5 ^a	—

^a Rejected parameters due to traction device breakages

Table 2. Mechanical test, hydroxy-apatite (HAP) results

Time (weeks)	Sample	Cortical bone width (mm)	Peak load in newtons (N)	Shear strength in megapascals (Mpa)
2	1	2.4	271.4	12.00
2	2	2.65	218.1	8.73
2	3	2.9	432.1	15.81
2	4	2.65	238.4	9.55
4	1	3.1	474.7	16.25
4	2	2.85	473.6	17.63
4	3	4.2	288.7	7.29
4	4	6.2	561.6	9.61
6	1	2.6	716.7	31.08
6	2	2.6	965.7	39.41
6	3	2.5	764.6	32.45
6	4	2.9	510.1	18.66
8	1	2.9	1065.6	38.99
8	2	2.7	868.4	34.13
8	3	3.45	706.8	30.61
8	4	3.1	1215.1	41.59
12	1	3.11	979.9	33.42
12	2	3.11	1162.6	39.65
12	3	3.11	1249.1	42.6
12	4	3.11	1274.0	43.45
16	1	2.7	748.8	29.43
16	2	3.6	809.6	23.86
16	3	3.4	678.3	21.17
16	4	4.0	1152.1	30.56

the cylinders had been extracted and the periosteal, cortical, endosteal, and medullary areas of the bone examined. Sections were also cut parallel to the axis of the femoral diaphysis at the level of the zone of transition from cortex to medulla, where the greatest formation of new bone takes place, so as to enable detailed examination of this area.

The sections were placed onto slides, covered with polyethylene film, and were then left under compression for 24 h. The following day the film was removed and the sections were stained. Goldner's trichromatic stain was used to show up cell structure and von Kossa's to enable differentiation of the mineralisation of the trabeculae. With this technique, the trabeculae become black, whereas the HAP and bioglass stain grey.

Results

Both coatings were applied by the plasma-spray technique, and showed the typical pattern of droplets spread over the metallic surface. The bioglass coating presented a greater roughness, porosity and pore size compared with the HAP. The porosity level of the implants coated with bioglass, was 22.4%, four times greater than that obtained with an HAP coating (4.9%). The percentage of pores more than 50 µm in size was higher with bioglass (30%) than with HAP (20%). The results of the mechanical test for both bioglass and HAP are shown in Tables 1 and 2.

The average shearing force for implants coated with HAP was six times greater than for bioglass cratures at 2 and 4 weeks, ten times greater at 6 weeks, four times at 2 and 4 months, and five times at 3 months.

The findings for shear strength were studied statistically. The Student *t* test was used to compare the bioglass and hydroxiapatite groups for each peri-

Table 3. Variance in equality for every period of time ($P < 0.01$). The calculated *F* is less than Snédecor *F* ($P < 0.01$) for every period of time

Weeks	Bioglass (BG) variance	HAP variance	Calculated F^3	Snédecor <i>F</i>
2	1.42	7.6	5.42	29.46 ¹
4	2.42	19.7	8.14	99.17 ²
6	3.72	56.1	15.16	29.46
8	8.56	17.72	2.07	99.17
12	13.83	15.46	1.11	29.46
16	1.21	15.04	12.43	99.17

¹ See the Snédecor *F* table for 3 *df* for both variances, the major and the minor one

² See the Snédecor *F* table for 3 *df* for the major variance and 2 *df* for the minor one

³ Major variance divided by the minor one

od of time, and the variance in equality with the Snédecor *F* test (Table 3). The findings for the Student *t* test are shown in Table 4.

Histological examination of the bioglass interface shows three reasons why it is less well integrated with bone. Is surrounded by an interface of fibrous tissue with a macrophagic reaction to a foreign body. There is less formation of new bone, with a significant delay in maturation. No laminar arrangement of the trabeculae was visible until after 3 months, whereas with HAP the trabeculae were already mature at 4 weeks. Bioglass also showed only slight mineralisation of the osteoid even after 4 months compared to HAP.

There were no post operative infections.

A study of the modes of failure of the cylinders showed a strong difference between the two types of

Table 4. Contrast of the difference between shear strength mean values

Weeks	Bioglass mean values	HAP mean values	Calculated <i>t</i>	Student <i>t</i> <i>P</i> <0.05	Student <i>t</i> <i>P</i> <0.01
2	2	11.5	5.49	2.44 ¹ (S)	3.7 ¹ (S)
4	2.93	12.6	3.06	2.57 ² (S)	4.03 ² (NS)
6	3	30.3	3.54	2.44 (S)	3.7 (NS)
8	9	36.2	8.11	2.57 (S)	4.03 (S)
12	8.21	39.78	10.11	2.44 (S)	3.7 (S)
16	6.04	26.25	7.43	2.57 (S)	4.03 (S)

¹ See Student *t* table for 6 *df*² See the same table for 5 *df*

S=significant; NS=non-significant

implant. In those coated with HAP mechanical failure always occurred within the bone. After 4 weeks, this was visible to the naked eye, as the bone broke on extraction, and fragments remained attached to the implant when it was removed. This indicates a noteworthy capacity for osteointegration.

However, in implants with a bioglass coating, failure always occurred at the interface between bone and coating. The implants were removed without any break in the bone with some areas retaining coating and others bare of it.

The bioglass coating disappeared more swiftly than HAP, from 4 weeks onward. At 4 months the bioglass coating had come off uniformly, but the HAP coating showed some areas which appeared intact, while others were devoid of coating.

Discussion

Coating of the titanium surface with bioglass by the plasma-spray technique achieves a greater level of porosity and a larger pore size than coating with HAP. Thus, the area of contact with the bone is larger. Gabbi and Locardi [5] established that bioglass had these better physical and chemical characteristics for spraying at high temperature. We have shown that bioglass is less well integrated into bone. It is known from previous studies [9] that when bioglass comes into contact with living tissue a silicone gel forms at the interface from 9 days onwards, which leads us to believe that its surface porosity may disappear.

The site of failure of all HAP implants was within the bone indicating a strong link between bone and implant and between HAP coating and the titanium substrate. Bioglass, however, failed at the interface between bone and coating, indicating a weaker link with the bone.

The mechanical tests showed that the shearing force was between four and ten times greater for HAP. This mode of failure of bioglass explained by the histological demonstration of the appearance of an interface of fibrous tissue with macrophages and only slight formation of new trabeculae. With bioglass there was retarded maturation of newly-formed bone, which was poorly mineralised.

Implants coated with HAP achieved greater integration than those coated with bioglass (GSB formu-

la), probably because of the excessive amount of aluminium oxide contained in the latter. Greenspan [7] demonstrated in 1976 that if 3% aluminium oxide is added to bioglass, its capacity to unite with the bone is completely lost.

We used aluminon staining to demonstrate accumulation of aluminium at the interface between the bone and the bioglass implant. This may impede the mineralisation of the osteoid.

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