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Evaluation of the acceptance of glass in bone

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Six glasses in the SiO_2–Na_2O–CaO–P_2O_5–Al_2O_3–B_2O_3-system were implanted in rabbit tibia. The bone–implant interfaces were studied by scanning electron microscopy (SEM) and in a push-out test. In SEM it seems possible to distinguish between physical contact and chemical bonding between glass and bone. The measured push-out strength is about 0.5 MPa if no bone contact exists. If physical contact exists the push-out strength is 2–3 MPa. The push-out strength of titanium falls within these limits. Glasses, which on basis of the SEM study are concluded to chemically bond to bone, show push-out strengths of 16–23 MPa. Two non-bonding glasses are compared. One possesses only a silica-rich surface, whereas the other possesses a calcium phosphate-rich surface. Both develop a close contact with bone, but neither bonds chemically. There is no significant difference in their push-out strengths, which are comparable to that of titanium. Even if a calcium phosphate-rich layer forms at the glass surface, bonding may be reduced if Al_2O_3 is included in the glass composition. Further, a phosphate-free bioactive glass is compared with two phosphate-containing bioactive glasses. The phosphate-free glass bonds by incorporating phosphate from the body fluid into its surface. Push-out data indicate that this glass is not as firmly attached to bone as the phosphate-containing ones. The calcium phosphate layer formed is non-uniform, which might explain the lower bonding strength.

1. Introduction

Bioactive glasses adhere to bone through a chemical bond [1]. Hench [2] has described the bonding mechanism as a sequence of reactions between the glass and the surrounding fluid. Gross and Strunz [3] described the bonding from a biological point of view. The net result of the bonding reactions is the formation of a silica-rich layer, and on top of this a calcium phosphate-rich layer which is firmly attached to the bone.

The integration of an implanted material in bone is often studied histologically by optical microscopy or by scanning electron microscopy (SEM). The optical microscope is rapid and convenient for studying the biological response to the implant at different stages in the integration process. The result may be quantified as the proportions of fibrous tissue, osteoid and bone at the implant surface. It is not, however, possible to distinguish between physical contact and chemical bonding in the optical microscope. The most reliable way of establishing whether bonding exists or not is by mechanical testing. The obvious difficulty is to develop a reliable mechanical test. The main problems are to measure tensile strength and to determine the contact area.

If the shear strength is measured instead of the tensile strength, there is a risk that not only the pure adhesion is measured, but that there also is a contribution from mechanical interlocking due to the surface roughness. It is difficult to determine the contact area since the bone growth is often conducted up along the mantle surface of the implant.

Cook *et al.* [4] measured the interface shear strength for hydroxyapatite-coated and uncoated cylinders in dog femur. A trephine-type burr was used to prepare a flat cross-section at the endosteal surface. Hereby a flat supporting surface and a measurable contact area are achieved. However, since cylinders were used one cannot exclude a mechanical contribution by the surface roughness. Fujiu and Ogino [5] used conical implants (glass and apatite) and claimed that the mechanical contribution was avoided when the taper was 1/20 or more. They reported difficulties in measuring the contact area because of the osteoconductive effect. Nakamura *et al.* [6] developed a test in which the implant is a rectangular plate. Prior to testing, the bone with the implant is prepared to consist of two bone pieces held together by the implant. By loading the bone pieces in opposite directions the tensile strength can be measured. However, the contact area is difficult to measure and therefore only the failure load is reported.

It seems that many different methods are employed to determine the bone bonding strength of bioactive materials, and that no consensus exists. Consequently

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it is difficult to compare measured strengths reported by different workers. Despite this, simple push-out tests are valuable in developing bioactive materials, since it is often enough to establish whether or not a glass bonds chemically to bone. Recently the *in vivo* behaviour of a number of glasses in the SiO_2–Na_2O–CaO–P_2O_5–Al_2O_3–B_2O_3 system was studied. Using scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDXA), bone contact and bone bonding were distinguished [7]. In the present investigation one inert, two biocompatible and three bioactive glasses from that recent work are studied. Titanium is used as control. Conclusions based on SEM, regarding the acceptance of glass in bone, are compared with results from a simple push-out test.

2. Experimental procedure

The glass compositions are given in Table I. The raw materials used were SiO_2, Na_2CO_3, $CaCO_3$, $CaHPO_4 \cdot 2H_2O$, Al_2O_3 and H_3BO_3. The glasses were melted in a platinum crucible for 2–3 h at 1340–1410 °C and cast into a preheated graphite mould. Hereby conical implants with a length of 5 mm, a base diameter of 2.5 mm and a cone angle of 9° were obtained.

Six cones of each glass and of titanium were implanted in rabbit tibia, the tip of the cones pointing to the marrow. Both legs were used. Prior to implantation the glasses were washed and sterilized ultrasonically in ethanol. The animals were anaesthesized. The bone preparation was done under sterile conditions using a burr for dental implants (Frialit) at approximately 700 r.p.m. under irrigation with 0.9% NaCl solution. The rabbits used were 4–7 months of age with a weight of 4–5.5 kg.

After 8 weeks the animals were sacrificed. The implants with surrounding bone were cut out as blocks. They were prepared for a push-out test by removing the bone covering the base of the cones by grinding. The base of the cone was also slightly ground together with the surrounding bone, to produce a flat supporting surface. Prior to testing, the samples were kept in a physiological saline solution at room temperature. Each test was performed within 6 h of the decapitation of the rabbit. The retention was measured by pushing five cones of each glass out of the bone at a rate of 0.5 mm min^{-1}. Fig. 1 shows schematically the push-out test fixture. The thickness of the cortex was measured for each sample. Due to experimental difficulties in measuring the contact area, only the "normal" thickness of the cortex was measured. Because of the osteoconductivity the contact area is somewhat underestimated (0–20%). After the push-out test, the specimens were fixed in buffered formaldehyde, dehydrated, and embedded in methylmethacrylate. For each material, one cone which was not push-out tested was sectioned along its axis. These sections were coated with carbon and studied with SEM. The accelerating voltage was 20 kV. Histological sections 6–10 μm thick were prepared of the push-out tested specimens and studied in the optical microscope.

TABLE I Glass compositions by synthesis

Designation	Composition (wt %)					
	Na_2O	CaO	P_2O_5	B_2O_3	Al_2O_3	SiO_2
S65.5P1	17.00	13.00	1.00	1.00	2.50	65.50
S52P3	18.00	24.00	3.00	0.00	3.00	52.00
S52P8	25.00	12.00	8.00	0.50	2.50	52.00
S45P7	24.00	22.00	7.00	2.00	0.00	45.00
S46P0	26.00	25.00	0.00	2.00	1.00	46.00
S55.5P4	29.00	11.00	4.00	0.00	0.50	55.50

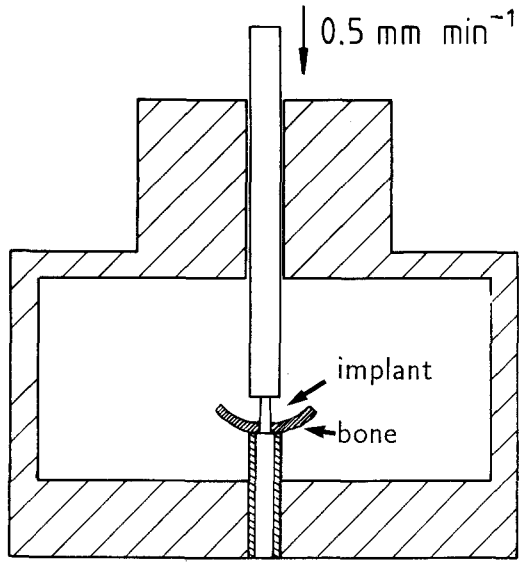


Figure 1 Schematic illustration of a specimen in the push-out test fixture.

3. Results and discussion

3.1. Push-out tests

On the basis of the push-out data the glasses fall into three groups. The first group only includes glass S65.5P1. This glass has a push-out strength of only 0.5 ± 0.4 MPa, which suggests that this glass is encapsulated in connective tissue.

Glasses S52P3 and S52P8 fall into the second group with push-out strengths of 3.6 ± 0.9 and 3.0 ± 0.5 MPa, respectively. This is considerably higher than for glass S65.5P1. Thus, the nature of the fixation of glasses S52P3 and S52P8 is different from that of glass S65.5P1. It may be expected that glasses S52P3 and S52P8 are surrounded by bone rather than by connective tissue. This is supported by the data for the titanium implants which show a push-out strength of 2.2 ± 0.6 MPa, i.e. not significantly different from that of glass S52P3 and S52P8. It is well established that titanium achieves a good bone contact.

The third group includes glasses S45P7, S46P0 and S55.5P4 with push-out strengths of 23.0 ± 2.9, 16.4 ± 3.9 and 19.9 ± 4.0, respectively. Clearly, these are far above what is expected for a material like titanium which only achieves close contact. Thus,

these glasses can be expected to chemically bond, i.e. to be bioactive. Glass S46P0 does not contain phosphate and also seems not to be as firmly attached to bone as the phosphate-containing glasses S45P7 and S55.5P4. That glass S46P0 is chemically bonded, however, is clear. This was recently shown [8, 9] and is also demonstrated in Fig. 2. This shows an optical micrograph of glass S46P0 after the push-out. It can be seen that the fracture occurs at least partly in the bone, whereas bone still is adhering to the glass.

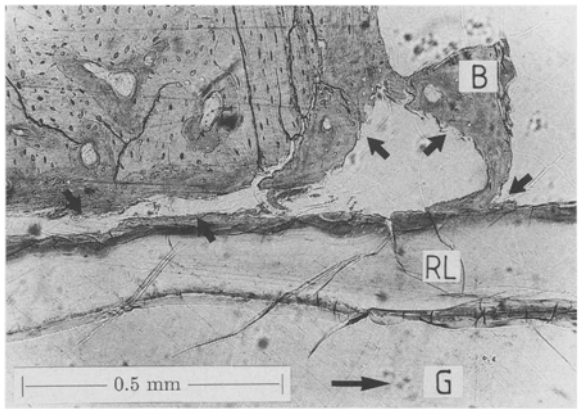


Figure 2 Optical micrograph of glass S46P0 after the push-out. Fracture occurs in the bone (B), whereas bone is still adhering to the glass (RL = reaction layer, G = bulk glass). Long arrow indicates direction of loading and short arrows the fracture line.

3.2. SEM study

The implants were studied by SEM in the back-scatter mode. In this way the formation of silica-rich (dark) and calcium phosphate-rich layers (light) is easily detected. Glass S65.5P1 (first group) which showed a very low push-out strength has hardly undergone any reactions (Fig. 3). Only a very thin silica rich layer forms. This is due to high SiO_2 and Al_2O_3 contents which increase the stability of the glass. Fig. 3 shows a selected spot where bone and glass are fairly close. However, most of the glass surface has a distance of 10–20 μm to the bone.

The push-out test indicated that glasses S52P3 and S52P8 (second group) develop a close contact to bone but do not bond. This is also the conclusion of the SEM study. A small gap or crack is seen between bone and glass. Presumably this gap was created during sample preparation. These glasses undergo extensive reactions. Glass S52P3 exhibits a fairly thick silica-rich (dark) layer (Fig. 4), but there are no signs of calcium phosphate formation at the surface. Recently it was found that Al_2O_3 is enriched at the surface of this glass *in vivo* [7]. This enrichment interferes with the bonding of calcium phosphate to the silica.

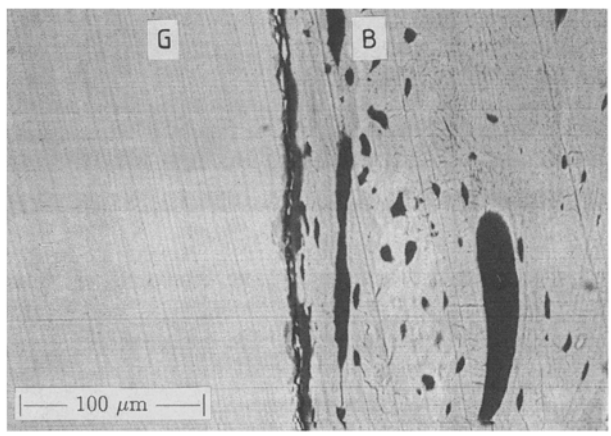


Figure 3 SEM micrograph of glass S65.5P1 showing poor or no contact after 8 weeks in rabbit tibia. G = bulk glass, B = bone.

Glass S52P8 differs from glass S52P3 in that a calcium phosphate-rich surface layer forms on the silica rich layer (Fig. 5). Both glasses have high Al_2O_3 contents. For glass S52P3 the calcium phosphate formation was presumably inhibited by Al_2O_3 whereas this is not the case for glass S52P8. This is explained by the lower CaO content in glass S52P8, which results in a higher solubility. The higher the solubility, the more Al_2O_3 can be added to the glass without inhibiting the calcium phosphate formation [7, 8]. The lack of bonding could be due to adsorption of Al^{3+} on the calcium phosphate surface or to aluminium-release into the tissue. For titanium (Fig. 6) a close contact to bone develops but no bonding occurs.

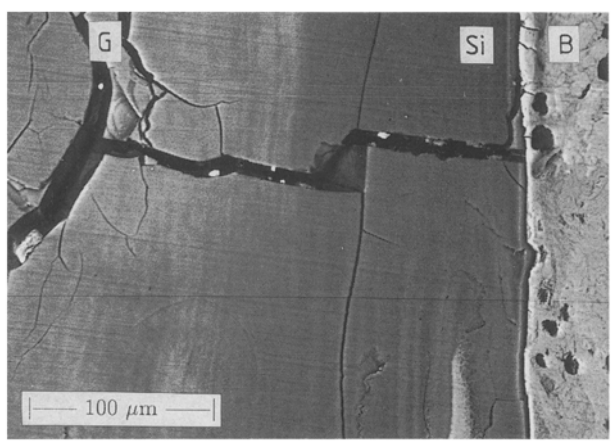


Figure 4 SEM micrograph of glass S52P3 showing contact, but no bonding, after 8 weeks in rabbit tibia, G = bulk glass, Si = silica-rich layer, B = bone.

The glasses in the third group, S45P7, S46P0 and S55.5P4, show considerably higher push-out strengths than the rest of the glasses in this study. Of these S45P7 and S55.5P4 are regular phosphate-containing bioactive glasses showing formation of a silica-rich and a calcium phosphate-rich layer (cf. glass S55.5P4, Fig. 7). As seen in Fig. 7, no gap can be seen between bone and glass. This is also the case for glass S46P0 (Fig. 8). Although this is a phosphate-free glass a calcium phosphate surface layer forms. The calcium phosphate accumulation takes place within the silica structure. Thus, phosphate from the surroundings penetrates into the glass surface. This phenomenon was discussed recently [9]. However, in Fig. 8 it can be seen that the calcium phosphate-rich layer is non-uniform. There are regions where there seems to be only a silica-rich surface and there are regions where a calcium phosphate surface has formed. The reason for

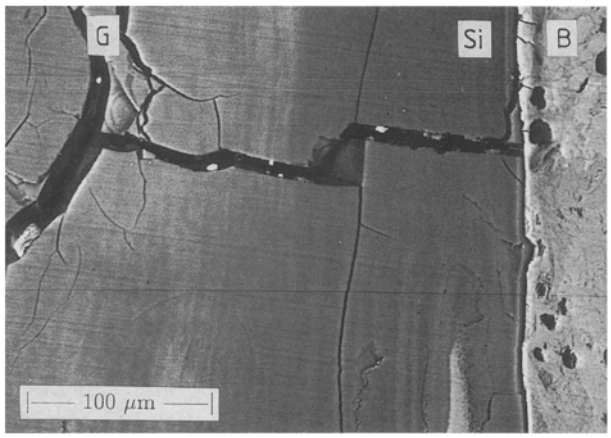


Figure 5 SEM micrograph of glass S52P8 showing contact, but no bonding, after 8 weeks in rabbit tibia. G = bulk glass, Si = silica-rich layer, CP = calcium phosphate-rich layer, B = bone.

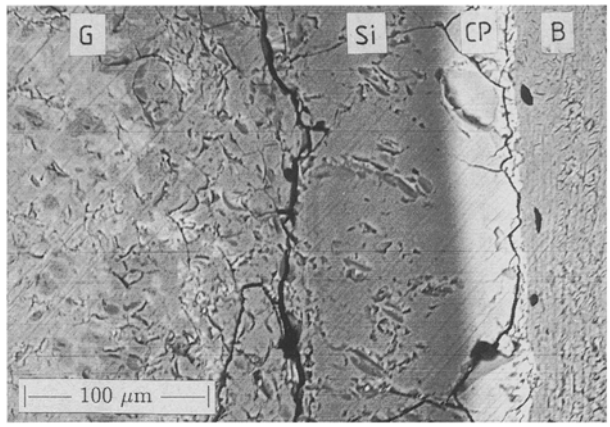


Figure 7 SEM micrograph of glass S55.5P4 showing bonding after 8 weeks in rabbit tibia. G = bulk glass, Si = silica-rich layer, CP = calcium phosphate-rich layer, B = bone.

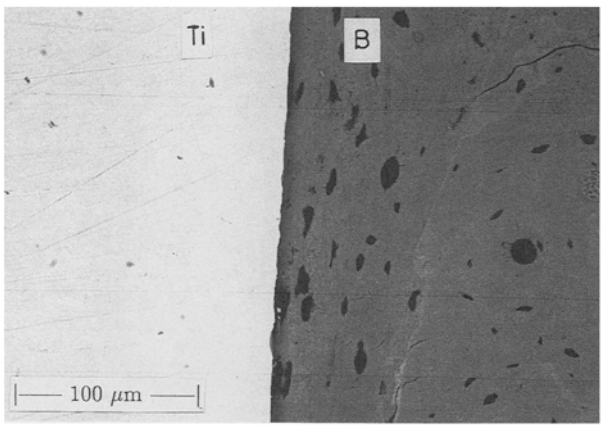
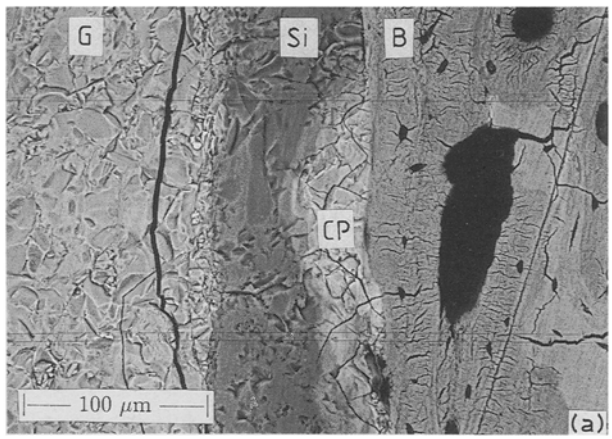


Figure 6 SEM micrograph of titanium cone showing contact, but no bonding, after 8 weeks in rabbit tibia. Ti = titanium, B = bone.



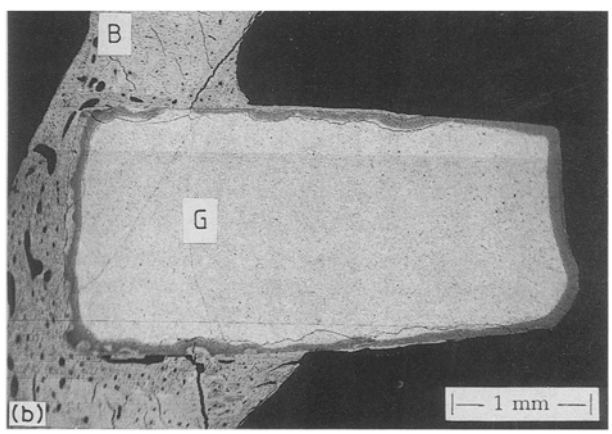


Figure 8 (a, b) SEM micrographs of the phosphate-free glass S46P0 showing bonding after 8 weeks in rabbit tibia. Phosphate from the surroundings has penetrated into the glass surface and a calcium phosphate surface has formed. The calcium phosphate-rich layer is non-uniform. G = bulk glass, Si = silica-rich layer, CP = calcium phosphate-rich layer, B = bone.

this is not known. However, the push-out test indicated a lower strength for this glass than for the other bioactive glasses. This can be explained by the non-uniformity of the calcium phosphate layer for glass S46P0.

4. Discussion

Table II summarizes the *in vivo* reactions, i.e. formation of silica-rich layer, formation of calcium phosphate-rich layer, bone response and push-out strength. In a previous work 16 silicate glasses of different compositions were studied and a classification was done according to their *in vivo* behaviour [7]. The groups are as follows:

Group A. Nearly inert glasses: only small changes in the surface composition and hardly any bone contact.

Group B. Fairly high solubility glasses: bone contact but no bonding; formation of a silica-rich layer but no calcium phosphate accumulation.

Group C. Fairly high solubility glasses: bone contact but no bonding; formation of a silica-rich (or Na-depleted) layer; limited calcium phosphate accumulation due to stabilization of the silica structure.

Group D. Fairly high solubility glasses: bone contact but no bonding; formation of a silica-rich and a calcium phosphate-rich layer.

Group E. Bioactive glasses: bone bonding; formation of a silica-rich and a calcium phosphate-rich layer.

TABLE II Summary of *in vivo* behaviour and push-out strengths

Sample	Si layer	Ca, P layer	Bone response (SEM)	Reaction type	Number of specimens	Strength (MPa)
S65.5P1	Thin	No	None	A	5	0.5 ± 0.4
S52P3	Yes[a]	No	Contact	B	4	3.6 ± 0.9
S52P8	Yes	Yes	Contact	D	5	3.0 ± 0.5
S45P7	Yes	Yes	Bonding	E	5	23.0 ± 2.9
S46P0	Yes	Yes	Bonding	E	4	16.4 ± 3.9
S55.5P4	Yes	Yes	Bonding	E	4	19.9 ± 4.0
Titanium			Contact		5	2.2 ± 0.6

[a] Al_2O_3 enrichment.

The groups were assigned numbers (A = 1, B = 2, C = 3, D = 4, E = 6). A model was then developed describing the relationship between glass composition and these reaction numbers (R.N.), i.e. the *in vivo* behaviour [7].

Glass S65.5P1 is of type A, glass S52P3 of type B, glass S52P8 of type D and glasses S45P7, S46P0 and S55.5P4 of type E. There exists a good qualitative agreement between the classification (no contact, contact, bonding) and the push-out data. The reaction numbers, however, do not correspond quantitatively to the push-out strength. The reaction numbers correspond to the reactivity (solubility) and increase with it. When the reactivity becomes high enough (R. N. = 5 to 6) a bone-bonding apatite surface forms. This results in a dramatic increase in the bonding strength. Thus, in the inert-to-bioactive reactivity range the push-out strength does not increase smoothly, but stepwise with increased reactivity.

In the bioactive range of compositions a higher reactivity results in a more rapid bone bonding. Hench [10] defined a bioactivity index, I_B. The basis for this index is the time for 50% of the implant surface to be bonded to bone. The more rapid the bonding, the higher the I_B value. However, as discussed by Hench [11], a high reactivity (high I_B value) results in a very thick silica-rich layer with a fairly low shear strength. In contrast, the A/W glass–ceramic [12] has a lower I_B value but a very high shear strength. Thus, the strength does not correspond to the level of bioactivity as defined by the I_B value. Therefore, if the reactivity of a bioactive material (or the composition) is to be quantitatively related to the integration of the material in bone, mechanical testing does not seem to be very useful.

Histological determination of the relative proportions of connective tissue, osteoid and bone as a function of implantation time probably gives a better understanding of the factors important for bone bonding. This approach is, however, only useful if the materials compared are known to be bioactive. If they are biocompatible like glasses of S52P3 and S52P8 or titanium, the results might be misinterpreted since bone bonding and bone contact cannot be distinguished histologically. Therefore, histological comparison between glasses in the inert-to-bioactive range is difficult. By SEM or a simple push-out test it can be established whether the material bonds or not. Once the material has been shown to bond, histological evaluation may be used to determine how much of the implant surface is bonded to bone.

Glasses S52P3 and S52P8 have fairly high Al_2O_3 contents. In the less soluble glass S52P3, the presence of Al_2O_3 inhibits calcium phosphate formation, whereas it does not in the more soluble glass S52P8. Thus, glass S52P3 develops only a silica-rich surface, whereas for glass S52P8 the silica-rich layer is covered by a calcium phosphate-rich one. However, there is no significant difference in the push-out strength between these glasses. Thus, the surface composition does not in this case affect the fixation of these materials in bone. The fixation of these biocompatible non-bonding glasses is similar to that of titanium.

4. Conclusions

The differences in push-out strength between no contact, contact, and bonding, are considerable. Thus, a simple push-out test can be used to assess the acceptance of a material in bone and relate it to other materials tested in the same way. Since only relative values are obtained, control specimens like titanium should be included.

The measured push-out strengths agree well with SEM observations. Thus, it seems possible by using SEM to qualitatively distinguish between bonding and non-bonding glasses without mechanical testing of the interface.

Glasses which contain Al_2O_3 might not bond to bone even if a calcium phosphate-rich surface layer forms. Therefore, the formation of a calcium phosphate-rich surface *in vivo* is not a sufficient criterion for bone bonding.

Biocompatible, non-bonding glasses which possess either a silica-rich or a calcium phosphate-rich surface have nearly equal push-out strengths. The push-out strength for titanium is comparable to that of these glasses.

In the inert-to-bioactive reactivity range the push-out strength does not increase smoothly, but stepwise with increased reactivity. By SEM or a simple push-out test it can be established whether the material bonds or not. For materials that have been shown to bond, histological evaluation may be used to determine the percentage of bone bonding and thus to

quantitatively correlate the biological response to composition or reactivity of the material.

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