Biomechanical assessment of bone ingrowth in porous hydroxyapatite

K. A. HING*, S. M. BEST, K. E. TANNER, W. BONFIELD
IRC in Biomedical Materials, Queen Mary and Westfield College, Mile End Road, London E1 4NS, UK

P. A. REVELL
IRC in Biomedical Materials, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3, UK

Porous hydroxyapatite (Endobon®) specimens were implanted into the femoral condyle of New Zealand White rabbits for up to 6 months. After sacrifice, specimens were sectioned for histology and mechanical testing, where the extent of reinforcement by bony ingrowth was assessed by compression testing and fixation was assessed by push-out testing. From histological observations, it was established that the majority of bone ingrowth occurred between 10 days and 5 weeks after implantation and proceeded predominantly from the deep end of the trephined defect, with some integration from the circumferential sides. At 3 months, the implants were fully integrated, exhibiting bony ingrowth, vascularization and bone marrow stroma within the internal macropores. After 5 weeks, the mean ultimate compressive strength of retrieved implants (6.9 MPa) was found to be greater than that of the original implant (2.2 MPa), and by 3 months the fully integrated implants attained a compressive strength of approximately 20 MPa. Push-out testing demonstrated that after 5 weeks *in vivo*, the interfacial shear strength reached 3.2 MPa, increasing to 7.3 MPa at 3 and 6 months.

1. Introduction

There has been much interest in the development of porous synthetic bone replacement materials for the filling of non-load-bearing osseous defects since the demonstration of improved biocompatibility in macroporous materials as compared with dense bodies [1, 2]. Hydroxyapatite (HA) has a similar crystal structure to that of bone mineral [3], and has been investigated as a bone replacement material for over 30 years [4–8]. It is generally acknowledged that HA is biocompatible and it has also been reported to exhibit osseoconductive properties, where osseoconduction is the ability of a material to encourage bone growth along its surface when placed in the vicinity of viable bone or differentiated bone-forming cells [9–12]. This property has led to numerous *in vivo* investigations of porous hydroxyapatite [13–17]. However, while the biological response to such materials is often reported, few studies consider these results in association with the mechanical characteristics of the implant–bone system after implantation. The objective of this investigation was to assess both the histological response and the reinforcing and fixating effects of progressive amounts of bone ingrowth within a porous hydroxyapatite implant, with time. The use of compression testing to evaluate the mechanical properties of cancellous bone is well documented and has been successfully applied to the testing of candidate synthetic bone materials in the as-received and post-implantation condition [13, 18, 19]. For assessment of implant fixation, push-out testing has been demonstrated to provide a simple method for the comparative assessment of the development of interfacial shear strength, with time, between host tissue and implant [11, 16, 20–22].

2. Materials and methods

2.1. Implant materials

In this study all specimens were composed of a commercially available porous HA, Endobon®, with a mean apparent density of $0.61 \pm 0.04\ g\,cm^{-3}$. Specimens were supplied in the form of cylinders with a mean diameter of 4.58 ± 0.06 mm, which were filed down to a length of 6.55 ± 0.58 mm. Comprehensive chemical characterization of the material has been previously reported, where it was determined that Endobon is composed of a partially carbonate-substituted hydroxyapatite containing minor ionic impurities as a result of the biological origin [23].

*Author to whom all correspondence should be addressed.
Selected paper from the 13th European Conference on Biomaterials, Göteborg, Sweden.

2.2. Implantation procedure and histological evaluation

Specimens were heat sterilized at 200 °C before implantation for periods of 10 days, 5 weeks, 3 and 6 months. Specimens were press-fitted into defects prepared using a saline-cooled, diamond-tipped trephine in the femoral condyle of penned New Zealand White rabbits. After sacrifice, sections were prepared for histology using the Exakt technique [24] and stained with toluidine blue. Histomorphometry was performed using point counting and linear intercept techniques. The percentage of bony ingrowth was measured using a Weibel grid composed of 42 points [25], from which, by measurement of both the total pore area available for osseointegration and the total area occupied by bone ingrowth within each section, the normalized percentage of bone ingrowth within the pore space for each implant was calculated [26]. The bone coverage over the implant surfaces was measured with a Merz grid [27] and calculated as the percentage of Endobon® pore surface occupied by bony ingrowth [26].

2.3. Mechanical testing

All mechanical testing was performed using an Instron 4464 bench top test machine fitted with a 2 kN load cell.

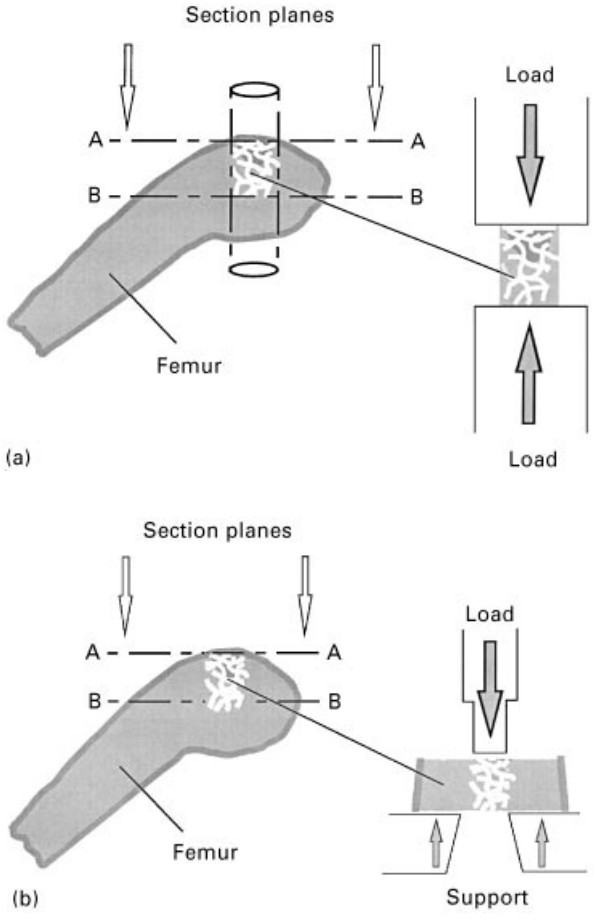


Figure 1 Preparation of test pieces from retrieved femora for (a) compression and (b) push-out testing.

2.3.1. Compression testing

The extent of reinforcement was assessed by compression testing of plugs trephined from retrieved femora (Fig. 1a), such that each test piece was composed of an intact implant with its associated bone ingrowth. Compression testing was carried out using an environmental chamber which allowed the test to be performed in Ringer's solution at 37 °C while load was applied axially to the specimens with a crosshead velocity of 0.1 mm min^{-1}.

2.3.2. Push-out testing

Fixation was assessed by measurement of the interfacial shear strength (ISS) using push-out testing. Tests were performed on sections of retrieved bone cut to expose the flat ends of the implants (Fig. 1b), using a specially designed jig (Fig. 2) to ensure the correct application of force. Load was applied at a crosshead velocity of 0.5 mm min^{-1} and prior to testing, specimens were soaked in Ringer's solution at 37 °C for 5 min.

3. Results

3.1. Histological evaluation

Ten days after implantation, little or no bone ingrowth was observed within the porous structure of the implants, with only minimal bone on-growth noted towards the specimen outer surfaces. However, there was evidence of considerable regeneration of bone around the edges of the defect in response to the surgical trauma and the porous structure appeared to have been infiltrated with mesenchymal cells and loose fibrous tissue (Fig. 3). Extensive osseointegration was noted at 5 weeks, with seams of osteoblasts depositing bone directly on the implant surfaces and the primary direction of bone ingrowth occurring from the deep end towards the superficial end of the defect (Fig. 4). From the results of the histomorphometry, it

Figure 2 Assembly for push-out testing.

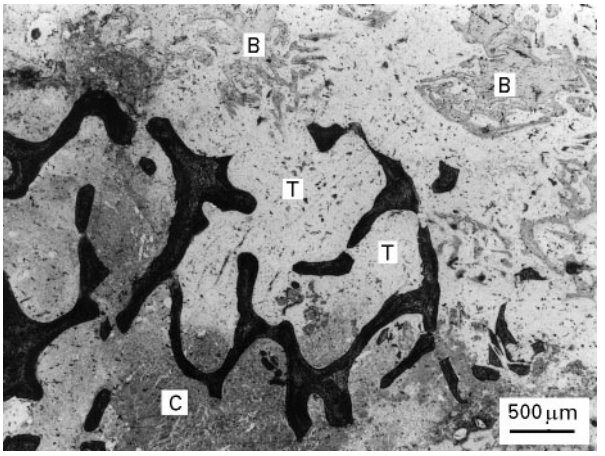


Figure 3 Longitudinal section through a porous HA implant 10 days after implantation demonstrating bone regeneration at the edges of the implant (B), blood clotting (C), mesenchyme and loose tissue (T) invasion.

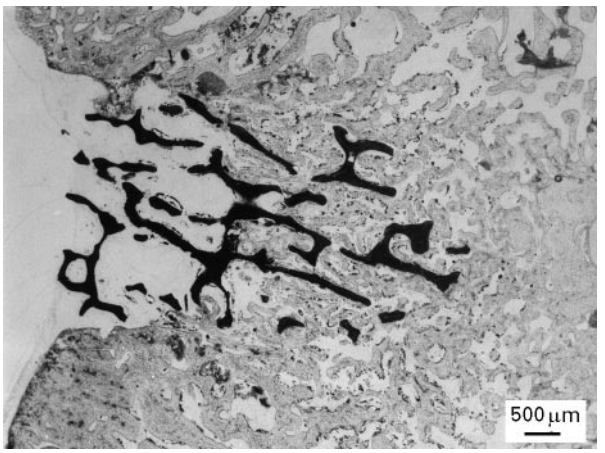


Figure 4 Longitudinal section through a porous HA implant 5 weeks after implantation demonstrating the ingress of bone from the deep end of the implant.

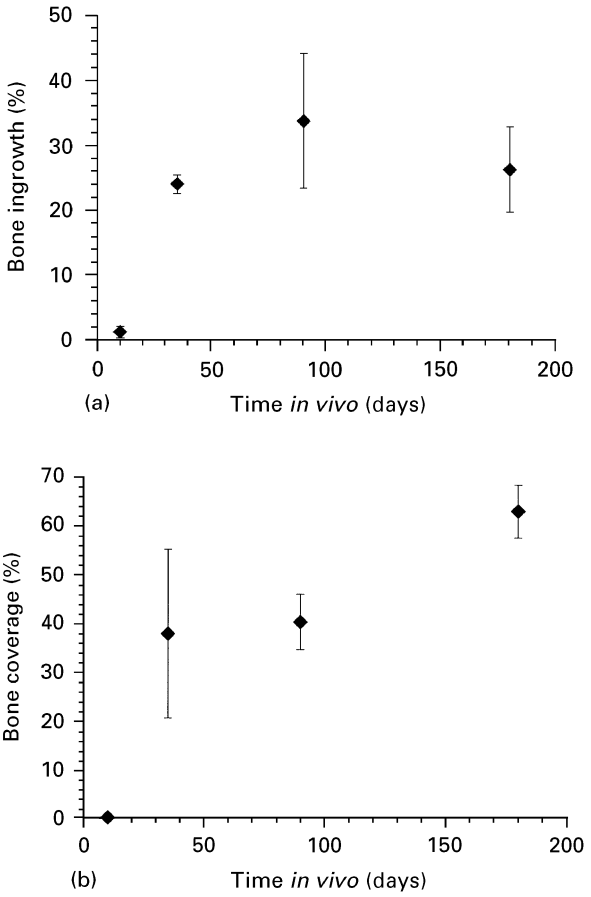


Figure 5 Variation of (a) the percentage of bone ingrowth within the internal macropore spaces, and (b) the percentage of internal macropore surface covered by bony ingrowth, with time *in vivo*.

was established that the majority of bone ingrowth occurred within the 10 days–5 weeks period, with full infiltration of cancellous bone throughout the macroporosity of the implants achieved by 3 months (Fig. 5a). The majority of bone surfaces were populated with cuboidal, darkly stained osteoblasts, indicating a high degree of activity in these cells (Fig. 6a). Furthermore, revascularization was also evident within the porous structure (Fig. 6b) at this time. There was little quantitative change in bone ingrowth between 3 and 6 months implants (Fig. 5a), but the percentage of bone coverage on the implant surfaces (internal and external) continued to increase up to 6 months post-implantation (Fig. 5b), consistent with the osteoblastic activity observed at 3 months (Fig. 6a). In contrast, the cells on the surfaces of the bony ingrowth at 6 months had the more quiescent appearance of bone lining cells, indicating that equilibrium had been reached (Fig. 7). Fibrous encapsulation was not noted at any time, and healthy osteocytes were found in close proximity to the surface of the Endobon® struts (Fig. 8). It was also noted that bony ingrowth tended to be sited on the hydroxyapatite pore surfaces rather than free standing within the macropores (Fig. 9a). However, the pore surfaces were not entirely covered by bone, with portions of the implant exposed directly to the bone marrow (Fig. 9b).

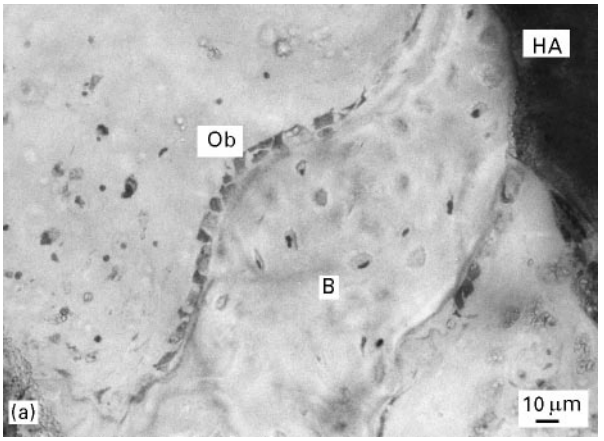
3.2. Mechanical testing

3.2.1. Compression testing

Both the compressive strength (Fig. 10a) and the compressive modulus (Fig. 10b) were found to increase with time *in vivo* up to 3 months, at which point peak values of approximately 20 MPa and 0.4 GPa, respectively, were achieved.

3.2.2. Push-out testing

Pushout testing of retrieved implants immediately post-implantation demonstrated a frictional contribution of approximately 0.1 MPa to the interfacial shear stress (ISS) measurement. This value increased from around 1.0 MPa at 10 days to 3.1 MPa at 5 weeks, when failure occurred by clean push-out of the implant/ingrowth plug. The ISS continued to rise with time *in vivo* until 3 months, to a value of approximately 7.3 MPa (Fig. 11). However, failure at this and later times occurred via fracture of implant/bone plug into three or more pieces, as opposed to failure at the interface (Fig. 12).



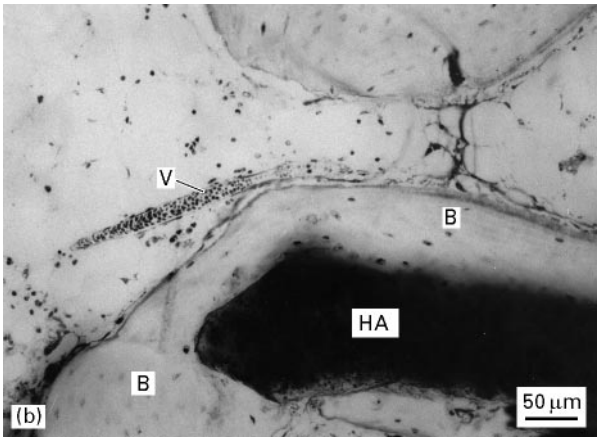


Figure 6 (a) Typical active osteoblasts (Ob) laying down bone within an internal macropore and (b) evidence of revascularization (V) occurring within porous hydroxyapatite (HA) implants after 3 months *in vivo*.

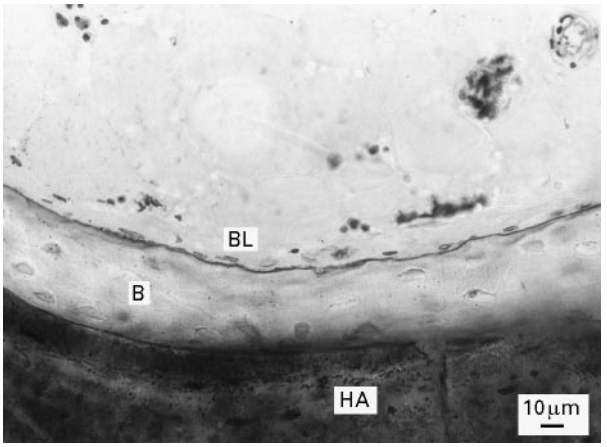


Figure 7 Quiescent bone lining cells (BL) on the surface of bone ingrowth (B) 6 months after implantation.

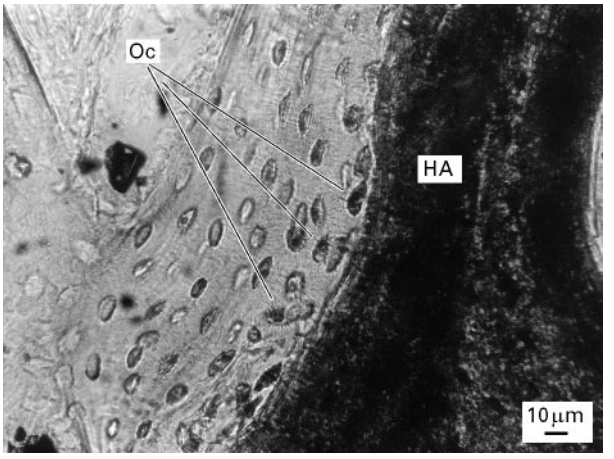
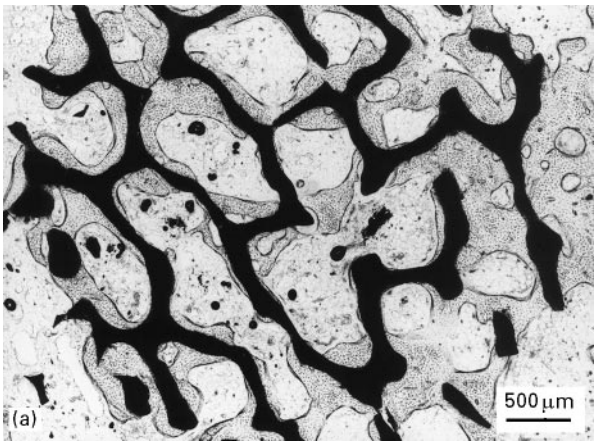


Figure 8 Proximity of healthy osteocytes (Oc) to the hydroxyapatite (HA) surface.



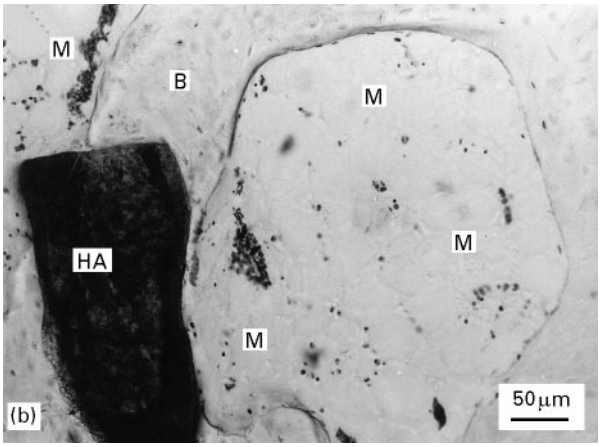
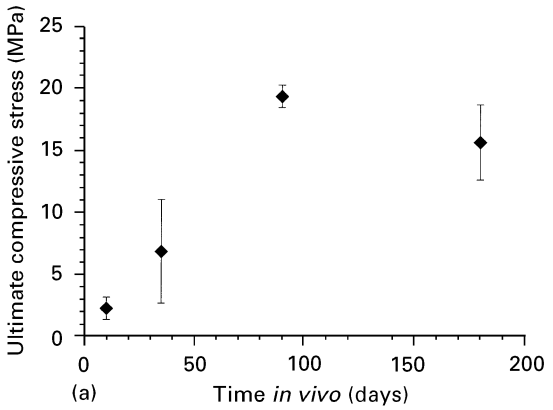


Figure 9 Transverse cross-section through a porous HA implant 6 months after implantation demonstrating (a) the distribution of bone ingrowth within the implant macroporosity and (b) direct contact of bone (B) and marrow (M) with the hydroxyapatite surface.

4. Discussion

Ideally, an implant, when placed in an osseous defect, should induce a response similar to that of fracture healing, whereby the defect is initially filled with a blood clot which is invaded by mesenchymal cells, osteoblasts and fibroblasts within 2 weeks, followed by extensive bone and osteoid formation at 6 weeks, with complete healing/repair of the cancellous structure by 12 weeks [17, 28]. This sequence of events broadly describes those observed within the Endobon® implants reflecting the biocompatibility of the material. However, where bone was reported to ingress primarily from the edges inward within an unfilled cavity [17], two distinct sequences were observed in the Endobon® implants. Observations at 10 days, indicate that bulk implants were initially osseoconductive, with rapid deposition of woven bone observed initiating from the defect walls towards the



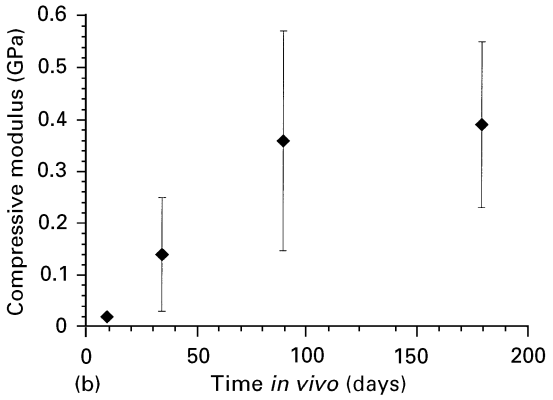


Figure 10 The variation of (a) ultimate compressive stress and (b) compressive modulus, with time *in vivo*.

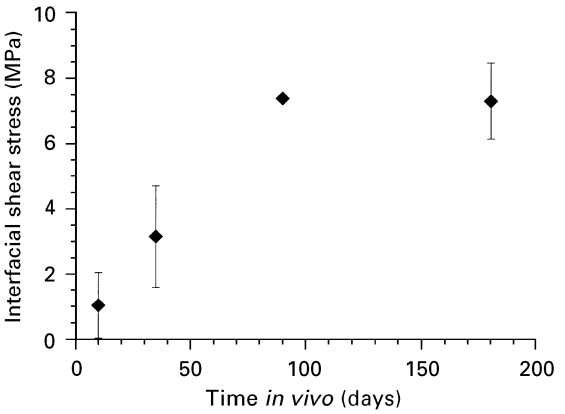


Figure 11 Results of push-out testing demonstrating the increase in interfacial shear stress between the integrated porous HA implant and the surrounding host tissue, with time *in vivo*.

implant (Fig. 3). After this initial period, once bone apposition and fixation had occurred, the Endobon® implant appeared to induce a more orderly deposition of lamella bone, both on the internal pore surfaces and within its pores, which appeared to advance from the deep end of the defect (Fig. 4), i.e. from the most abundant source of potentially osteogenic cells. A retarded version of these events was observed within coral-derived porous hydroxyapatite implants, although accelerated bone growth from the deep end of the implant was not reported [17]. In contrast, other investigations [15, 16] have reported incomplete penetration of bone into the centre of porous HA implants, possibly as a result of inhibited revascularization due to poor connectivity between

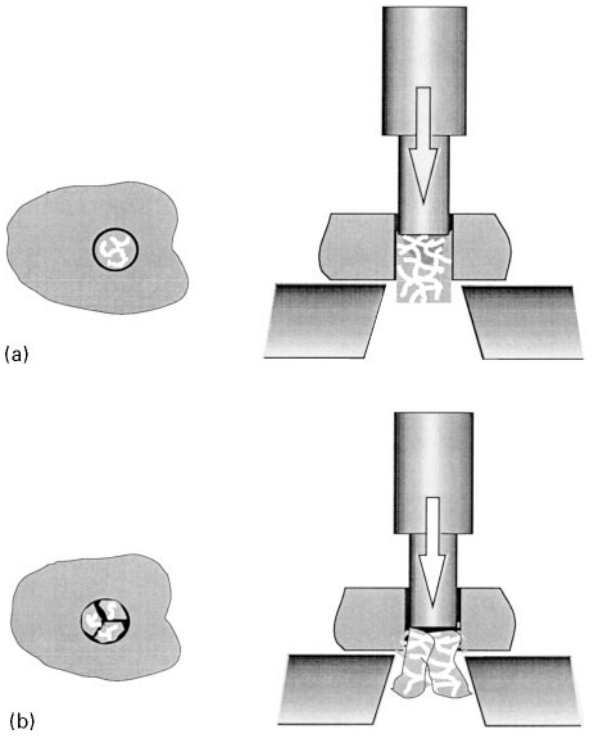


Figure 12 Schematic diagram illustrating the change in failure mechanism during push-out testing between implants tested (a) within 5 weeks and (b) after 3 months, of implantation.

the macropores. Furthermore, coralline porous HA was reported [13] to elicit macrophage and osteoclast activity on the implant surfaces up to 6 months after implantation, which was not observed on the Endobon® implant surfaces. However, healthy osteocytes with many canaliculi were evident in close proximity to the bone–Endobon® interface (Fig. 8).

The capacity of the newly formed bone to enhance significantly the mechanical performance of the implant after 5 weeks *in situ*, with a 300% increase in compressive strength, despite incomplete osseointegration at this time, demonstrated the strong reinforcing effect of the bone ingrowth (Fig. 10). Furthermore, both the compressive strength and the percentage of ingrowth, were found to follow a similar trend with time after implantation, where both variables attained a maximum value after 3 months (Figs 5a, 10a). The change in the activity of the cells on the bony ingrowth surfaces, observed between 3 and 6 months, indicated the attainment of an equilibrium by 6 months. However, while both the percentage of bone ingrowth and the mechanical properties demonstrated minimal variation over this period, a significant increase in bone coverage was noted. These findings suggested that remodelling occurred within the implant which was mediated by the local mechanical environment [29], indicating advantageous interaction between the integrated implant and the surrounding host tissue. A similar trend was reported for coral derived hydroxyapatite implants [18], where the mechanical properties did not vary significantly after 8 weeks *in vivo*, despite significant decreases in the percentage of bony ingrowth within the pore space of the implants, from 35% at 3 months to 17% at 1 year. This level of bone resorption was not observed within the Endobon®

implants at 6 months, neither was there significant osteoclastic activity on the trabecular surfaces.

Push-out testing of retrieved implants at 10 days demonstrated that, despite the minimal amount of bony ingrowth, there was still some degree of "bonding" between the implant and host tissue (Fig. 11). A significant portion of this bonding may have been due to mechanical interlock with the blood clot and loose fibrous tissue (Fig. 3), as the effect of friction was found to be minimal by comparison (0.1 MPa). Five weeks after implantation, the interfacial strength increased to approximately 3 MPa, a value which compared well with the values reported in the literature for porous HA cylinders [22]. Study of the failed test pieces indicated that specimens tested within 5 weeks *in vivo* had failed by extrusion of the specimen. However, at 3 and 6 months, the fracture mode was more disordered with failure occurring as a result of the implants being split longitudinally into three or more similarly sized portions during push-out (Fig. 12). These observations indicated that at 3 and 6 months post-operatively, the shear strength at the bone-implant interface exceeded the internal fracture strength of the osseointegrated implant. Furthermore, at 6 months, the implants tended to split into a greater number of pieces during failure, suggesting that the osseointegrated implant had achieved a greater degree of integration, which reflected the increase in bone coverage at this time (Fig. 5b). It is also interesting to note that the measured ISS for the implants was considerably reduced when compared to the ultimate compressive strength (UCS) of the implants at the same time point and that the shear strength of cancellous bone has been reported to be approximately 6 MPa [30].

5. Conclusion

This study demonstrates that Endobon® is highly biocompatible, with full osseointegration and vascularization achieved 3 months after implantation. Mechanical testing of the retrieved implants demonstrated a correlation between the degree of bony ingrowth, the compressive properties of the osseointegrated implant and the degree of fixation with the surrounding bone. Compressive strength was significantly enhanced by the presence of bony ingrowth, with a 300% increase in UCS, 5 weeks after implantation. Fixation also developed rapidly as a result of bony integration throughout the circumferential and deep macropores of the implant.

Acknowledgements

The authors gratefully acknowledge the Engineering and Physical Sciences Research Council (UK) for the funding of the IRC in Biomedical Materials, Heath Engineering (UK) for the design and manufacture of the push-out jig, and the support of E. Merck GmbH (Germany).

References

1. S. F. HULBERT, J. S. MORRISON and J. J. KLAWITTER, *J. Biomed. Mater. Res.* **6** (1972) 347.
2. J. J. KLAWITTER and S. F. HULBERT, *ibid.* **5** (1971) 161.
3. W. F. DE JONG, *Rec. Trav. Chim.* **45** (1926) 445.
4. H. W. DENISSEN, K. DE GROOT, A. A. DRIESSEN, J. G. C. WOLKE, J. G. J. PEELEN, H. J. A. VAN DIJK, A. P. GEHRING and P. J. KLOPPER, *Sci. Ceram.* **10** (1980) 63.
5. G. DE WITH, H. J. A. VAN DIJK, N. HATTU and K. PRIJS, *J. Mater. Sci.* **16** (1981) 1592.
6. M. AKAO, H. AOKI and K. KATO, *ibid.* 16 (1981) 809.
7. M. JARCHO, C. H. BOLEN, M. B. THOMAS, J. BOBICK, J. F. KAY and R. H. DOREMUS, *Ibid.* **11** (1976) 2027.
8. H. M. ROOTARE and R. G. CRAIG, *J. Oral Rehab.* **5** (1978) 293.
9. S. H. MAXIAN, J. P. ZAWADDSKY and M. G. DUNN, *J. Biomed. Mater. Res.* **28** (1994) 1311.
10. B. A. RAHN, J. NEFF, A. LEUTENEGGER, R. MATHYS and S. M. PERREN, in "Biological and Biomechanical Performance of Biomaterials", edited by P. Christel, A. Meunier, A. J. C. Lee (Elsevier Science, Amsterdam, 1986) p. 21.
11. P. K. STEPHENSON, M. A. R. FREEMAN, P. A. REVELL, J. GERMAN, M. TUKE and C. J. PIRIE, *J. Arthroplasty* **6** (1991) 51.
12. A. MORONI, V. J. CAJA, E. L. EGGER, L. TRINCHESE and E. Y. S. CHAO, *Biomaterials* **15** (1994) 926.
13. J. H. KÜHNE, R. BARTL, B. FRISH, C. HANMER, V. JANSSON and M. ZIMMER, *Acta Orthop. Scand.* **65** (1994) 246.
14. P. S. EGGLI, W. MULLER and R. K. SCHENK, *Clin. Orthop. Rel. Res.* **232** (1988) 127.
15. R. E. HOLMES, V. MOONEY, R. BUCHOLZ and A. TENCER, *ibid.* **188** (1984) 252.
16. W. RENOOIJ, H. A. HOOGENDOORN, W. J. VISSER, R. H. F. LENTFERINK, M. G. J. SCHMITZ, H. VAN IEPEREN, S. J. OLDENBURG, W. M. JANSSEN, L. M. A. AKKERMANS and P. WITTEBOL, *Ibid.* **197** (1985) 272.
17. H. LIN, H. XU and K. DE GROOT, *J. Biomed. Mater. Res.* **26** (1992) 7.
18. R. B. MARTIN, M. W. CHAPMAN, N. A. SHARKEY, S. L. ZISSIMOS and B. BAY, *Biomaterials* **14** (1993) 341.
19. M. TRÉCANT, J. DELÉCRIN, J. ROYER, E. GOYENVALLE and G. DACULSI, *Clin. Mater.* **15** (1994) 233.
20. W. J. A. DHERT, C. P. A. T. KLEIN, J. G. C. WOLKE, E. A. VAN DER VELDE, K. DE GROOT and P. M. ROZING, *J. Biomed. Mater. Res.* **25** (1991) 1183.
21. S. UETA, *Kitasato Med.* **22** (1992) 116.
22. K. E. TANNER, C. DOYLE and W. BONFIELD, *Adv. Biomater.* **9** (1990) 149.
23. K. A. HING, S. M. BEST, K. E. TANNER, P. A. REVELL, and W. BONFIELD, in "Bioceramics 8: Proceedings of the 8th International Symposium on Ceramics in Medicine", edited by J. Wilson, L. L. Hench and D. Greenspan (1995) p. 75.
24. K. DONATH, *J. Oral Pathol.* **11** (1982) 318.
25. E. R. WEIBEL and H. E. ELIAS, in "Quantitative Methods in Morphology" (Springer, Berlin, 1967).
26. K. A. HING, PhD Thesis, University of London (1996) p. 202.
27. W. A. MERZ and R. K. SCHENK, *Acta Anat.* **76** (1970) 1.
28. G. HEIMKE, in "Osseo-Intergrated Implants" (CRC Press, Boca Raton, FL, 1990) p. 2.
29. L. E. LANYON, *J. Biomech.* **20** (1987) 1083.
30. J. L. STONE, G. S. BEAUPRE and W. C. HAYES, *J.Biomech.* **16** (1983) 743.

Received 5 May
and accepted 12 May 1997