The interactions of bisphosphonates in solution and as coatings on hydroxyapatite with osteoblasts

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Aseptic loosening is one of the major causes of failure of artificial hip joints, and it can occur for several reasons, including osteolysis of the bone tissue in response to stress shielding or cellular reactions to wear debris. Any treatment of the prosthesis which could minimize the osteolytic response of the bone tissue may be able to extend the life-time of the implant. Bisphosphonates are potent inhibitors of osteoclastic bone resorption, and they bind avidly to hydroxyapatite (HA). Coating the prostheses with bisphosphonates may therefore inhibit osteolysis. We have investigated the potential for this approach by determining whether bisphosphonates interact with osteoblasts in vitro. The effects of pamidronate (P), clodronate (C), and etidronate (E) in solution and when coated onto HA were investigated. P inhibited protein and collagen syntheses potently when in solution, but not after being bound to HA. When bound to HA, both P and C increased DNA, protein and collagen syntheses of osteoblasts and may encourage the osseointegration of implants. The pharmacological effects of the bisphosphonates studied altered dramatically after binding to HA. This must be fully investigated before this approach to prolonging prostheses stability can be evaluated.


Introduction
There are currently around 500,000 artificial hips implanted annually worldwide [1], and increasing numbers of these are being implanted into younger patients. The life expectancy of artificial hips is limited, and few survive beyond 20 years. Thus, the need to develop artificial hip joints with extended life-times has become an important issue.

The most common cause of failure is aseptic loosening where, in the absence of infection, after a certain amount of wear, the fixation of the implant into the bone fails, and the artificial joint becomes loose. Aseptic loosening may occur for several reasons, including osteolysis of the bone tissue in response to stress shielding, osteolysis caused by cellular reactions to wear debris, and/or motion at the bone–cement–implant interfaces [2]. Factors such as surgical technique, initial stability and mechanical factors will also contribute. Any treatment of the prostheses which could minimize the osteolytic response of the bone tissue in response to both stress shielding or wear debris may be able to extend the life-time of the implant.

Bone remodeling occurs continuously in the skeleton by the co-ordinated actions of osteoblasts, which secrete new matrix, and osteoclasts, which resorb old bone. Any agent which reduces the efficiency of the later process will inhibit the osteolysis associated with prosthesis loosening. Bisphosphonates are the most effective inhibitors of osteoclastic bone resorption [3–10]. They are pyrophosphate analogs in which the oxygen in the P–O–P has been replaced by a carbon to yield a P–C–P backbone. Substitutions on the carbon yield a large family of compounds with different properties and potencies which are determined by nature of the side chains [11]. Bisphosphonates are used therapeutically in a variety of diseases of enhanced bone resorption, including Paget’s disease, hypercalcemia of malignancy, and osteoporosis, so there are data available on their

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efficacy, safety and pharmacodynamics in humans following both oral and intravenous administration [11]. Studies have suggested that the mechanism by which these compounds inhibit bone resorption is at a cellular level, affecting cells of the osteoclast lineage [3–10]. They have two modes of action on osteoclastic bone resorption, inhibiting both the acession (migration to the bone surface and fusion) of osteoclast precursors, and resorption by the mature osteoclast [3,6,8–10]. The latter occurs as a result of the bisphosphonates being internalized into osteoclasts by fluid-phase pinocytosis or by phagocytosis [9] and, once internalized, they affect a multitude of biochemical processes such as cell number, production of lactic acid [4], disruption of the osteoclast cytoskeleton [9], and prostaglandin production [7]. These result in a loss of the ability to resorb bone or even osteoclast cell death.

Bisphosphonates bind avidly to the bone mineral hydroxyapatite (HA), and in humans the drugs are released only slowly during skeletal remodeling. For example, the half-life of alendronate in humans is approximately 10 years [12]. Previous reports have shown that bisphosphonates may be immobilized on HA-modified titanium for dental applications [13]. We propose that a coating of bisphosphonate on the HA ceramic of artificial hip joints may function to inhibit osteolysis of the surrounding tissue, reduce the tendency towards loosening and, therefore, improve the life-time of the implant. However, it should be borne in mind that many of the bisphosphonates also inhibit mineralization, albeit at concentrations up to 1000-fold higher than those which inhibit resorption. The relative potency varies tremendously within the bisphosphonate drugs. Etidronate, for example, inhibits mineralization at doses very close to those which inhibit resorption [14], and it has been shown to inhibit the calcification of bioprosthetic heart valves. To take advantage of this property investigations are underway to bind bisphosphonates covalently to the valves, so there is a precedent for coating medical devices with bisphosphonates. To determine whether this approach is feasible for orthopedic implants, there are several questions which need to be addressed. In the first study aimed to answer these questions we have investigated the effect of bisphosphonates, both in solution and when coated onto HA material, on the viability, growth and function of osteoblasts. There is little information available on the interaction of bisphosphonates with osteoblasts. However, what is known, is that bisphosphonates have various inhibitory and stimulatory affects on the secretion and proliferation of osteoblasts which differ from one species to another [15–18]. In cultured human osteoblasts etidronate and pamidronate stimulate mineralization at concentrations below 10 nM, but are inhibitory above 1 μM [18]. 3H-Thymidine uptake into DNA is also inhibited by both of these drugs (10 nM) [17], and in addition, Igarashi et al. [16] found that bisphosphonates inhibited the proliferation of mouse osteoblasts at concentrations of 250 μM. Thus, although bisphosphonates have well-documented effects on bone resorption, their effects on bone formation may also be important. Although recent studies have shown that pamidronate immobilized on HA-coated titanium sur-

Materials and methods
Preparation of materials
Sodium clodronate ((C), Bonefos, Boehringer Ingelheim, Bracknell, Berkshire, UK), Etidronate ((E), Didronel, Procter & Gamble, Staines, UK) and Pamidronate ((P), Aredia, Ciba, Camberley, Surrey) were prepared as 0.22 M, 0.049 M, and 0.038 M solutions in serum-free medium respectively. The structures of these drugs are shown in Fig. 1.

To produce pellets of dense HA material (pore sizes < 3 μm) 55 g of calcium phosphate powder (tricalcium phosphate 34–4% Ca) was mixed with 10 ml of 4% (w/v) polyvinyl alcohol in distilled water. Using an Instron machine 1 g of powder was placed in a compact motor and pressure of 750 kg applied to produce 1 cm × 0.5 cm dense pellets. The samples were then sintered at 1150 °C for 2 h. After sintering, samples were polished using silicone carbide paper, and then cleaned for 1 h in 70% (v/v) alcohol, and then for a further 1 h in distilled water at 37 °C. A number of polished and cleaned samples of the material were then coated with 4 × 200 μl aliquots of either 3 mg/ml disodium clodronate or 1 mg/ml disodium pamidronate. The coated substrates were dried under a controlled.

Figure 1 Chemical structures of the sodium salts of (A) clodronate, (B) pamidronate and (C) etidronate.