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Growth of a bonelike apatite on chitosan microparticles after a calcium silicate treatment

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Abstract

Bioactive chitosan microparticles can be prepared successfully by treating them with a calcium silicate solution and then subsequently soaking them in simulated body fluid (SBF). Such a combination enables the development of bioactive microparticles that can be used for several applications in the medical field, including injectable biomaterial systems and tissue engineering carrier systems. Chitosan microparticles, 0.6 μm in average size, were soaked either for 12 h in fresh calcium silicate solution (condition I) or for 1 h in calcium silicate solution that had been aged for 24 h before use (condition II). Afterwards, they were dried in air at 60 °C for 24 h. The samples were then soaked in SBF for 1, 3 and 7 days. After the condition I calcium silicate treatment and the subsequent soaking in SBF, the microparticles formed a dense apatite layer after only 7 days of immersion, which is believed to be due to the formation of silanol (Si–OH) groups effective for apatite formation. For condition II, the microparticles successfully formed an apatite layer on their surfaces in SBF within only 1 day of immersion.

Keywords: Apatite; Biomimetic process; Calcium silicate; Chitosan; Microparticles

1. Introduction

Organic materials have surfaces which can be tailored to achieve different properties, such as hydrophilicity and the capability of carrying functional groups. In addition, they have a high degree of structural flexibility and may have strong surface-specific binding forces, such as the ability of their functional groups to chelate metal ions [1]. Therefore, new strategies aim at the surface modification of organic materials to obtain a biologically active surface while maintaining their bulk properties. A prime example of biomineralization would be a polymer matrix which can be placed into a metastable solution and induce precipitation to occur within the polymer but not in the solution [1]. For instance, the use of degradable polymers as matrices is a major approach in the development of materials for bone regeneration and replacement. However, biodegradable materials have to degrade without an unresolved inflammatory response or an extreme immunogenicity or cytotoxicity [2–4].

Chitosan, a linear polysaccharide, composed of glucosamine and *N*-acetyl glucosamine linked in a β(1–4) manner, is one of the most abundant natural polysaccharides, obtained from chitin by deacetylation [5–7]. The high content of primary amino groups gives chitosan unique properties, and in addition it is non-toxic, biocompatible and degradable, which makes it very attractive for clinical uses

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[5–10]. It also has good solubility in different organic acid solutions and sufficient resistance in alkaline solutions [11].

Synthetic biomaterials for tissue regeneration or replacement have to show physical and/or biological functions in intimate contact with hard or soft living tissue, but they are generally non-vital and bond poorly with the host tissue [12]. Therefore, the interface between the tissue and the implants is not strong, and the interface detaches easily under shear and distraction loads [13].

The biological responses of materials, such as bone bonding, are very important in clinical applications such as bone repair. For example, hydroxyapatite (HA), one of the major constituents of bones and teeth [4,14,15], has often been designated as an osteointegrating and/or osteoconductive (i.e. bioactive) material [16]. Bioactive HA ceramic in various forms has been used clinically during the last few decades because of its good biocompatibility [4,14,15] and efficacy in promoting biointegration for implants in both hard and soft tissue [17]. Moreover, the analysis of the interface between the bone and the bioactive implant is morphologically comparable to that of cement lines found naturally in bone remodelling sites, and this interfacial layer is formed on the chemically active surface of the biomaterial, which is one of the key features of the bonding zone and is unique to bioactive materials [18,19]. Such a type of calcium phosphate (Ca–P) layer is not observed around materials that are not bioactive, like metals and polymers, when used in bone defects, implying that the surface biomineralization of the bone mineral-like Ca–P is a precondition of bioactivity [12]. Furthermore, for bone replacement, a strong bonding between the host bone and the osteoconductive surface is also required [20–23]. Chitosan itself is not able to induce the formation of an apatite layer, i.e. it lacks bioactivity, even though it contains amino and hydroxyl groups.

Bioactivity can be induced on bioinert surfaces either by the formation of functional groups or by the formation of thin ceramic phases that have the potential to form functional groups on exposure to a body environment [24]. The key point lies in the design of a surface that is organized and functionalized so as to control the mechanisms of heterogeneous nucleation.

For instance, it has been demonstrated [25] that by using a simple biomimetic spraying methodology on chitosan fibre meshes, scaffolds, produced by wet-spinning, were able to induce the formation of a Ca–P layer when immersed in a simulated body fluid.

It has been reported that silanol (Si–OH) groups on polymeric materials can induce apatite formation in acellular simulated body fluids (SBF) with ion concentrations nearly identical to those of human blood plasma [26–29]. The incorporation of Si–OH groups into the chitosan microparticles can give bone-bonding ability to the microparticles, accelerating the tissue integration, which leads to the unique strength of such interfaces. In addition, their degradation rates make this material suitable as a bone substitute. Furthermore, chitosan microparticles can be easily handled during surgery, and such a mouldable visco-elastic material or injectable bone substitute is more easily applied than pure HA powder or granules and avoids the migration of HA particles [11,18,30–32].

The aim of this study was to incorporate Si–OH groups onto chitosan microparticles by soaking them in a calcium silicate solution, in order to obtain bioactive microparticles for designing an injectable bone substitute system.

2. Materials and methods

Chitosan (with a deacetylation degree of 87%) was obtained from Sigma Chemical Co. (St. Louis, MO). Acetic acid and cyclohexane were obtained from Merck (Darmstadt, Germany). Sodium sulphate was obtained from Aldrich Chemical Co. (Milwaukee, WI). All the other chemicals used were of analytical grade.

2.1. Preparation of chitosan microparticles

Chitosan microparticles were prepared by the water-in-oil/solvent evaporation method [33]. A 50 ml quantity of chitosan solution (0.25% (w/v), in 1% aqueous acetic acid) containing Tween-80 (0.5 ml) was prepared by using cyclohexane (75 ml) as the organic phase. Aqueous chitosan solution was emulsified with cyclohexane in a glass beaker (250 ml) by magnetic mixing at 1100 rpm until the formation of a milky solution (about 30 min). The stabilization of the chitosan droplets in the emulsion was achieved by ionic crosslinking of chitosan with sodium sulphate [33]. A sodium sulphate solution (10%, 5 ml) was added to the suspension in a dropwise manner. The amount of sodium sulphate needed for stabilization of chitosan microparticles was estimated visually by premixing sodium sulphate solution with chitosan solution without cyclohexane. The volume of sodium sulphate at which turbidity began was regarded as the ideal amount. Afterwards, the suspension was further stirred on a magnetic stirrer for 2 h at room temperature. Cyclohexane was removed from the emulsion by using either rotary evaporation or slow magnetic mixing of the suspension overnight. The chitosan particles formed were washed by centrifuging the suspension at 8000 rpm for 25 min using a clinical centrifuge. The microparticle pellet was resuspended with distilled water and centrifuged again. This procedure was repeated four times in order to wash the chitosan particles free from the emulsifying agent and excess sodium sulphate used in their preparation.

2.2. Calcium silicate solution treatment

In this study, the chitosan microparticles were freeze-dried to remove the water content before the calcium silicate solution treatment. Tetraethoxysilane (TEOS: $Si(OC_2H_5)_4$) (Nacalai Tesque Inc., Japan), ultrapure water, ethyl alcohol (C_2H_5OH) (Nacalai Tesque Inc., Japan), 1.0 M aqueous HCl solution and calcium chloride ($CaCl_2$) (Nacalai Tesque Inc., Japan) were mixed for 10 min at 0 °C

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