JOURNAL OF MATERIALS SCIENCE **39** (2004) 1029– 1032

Electrospraying of a nano-hydroxyapatite suspension

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Over the past two decades it has been established that bone repair in the presence of implant materials can be optimized by control of their surface chemistry. Certain calcium phosphate materials are similar in composition to bone mineral and have been found to be osteoconductive [1–3]. These ceramic materials have therefore been developed as biomaterials for a range of different applications. However, although they promote direct bone apposition, their mechanical properties are relatively poor compared with other monolithic ceramics. Therefore, classes of applications have been developed to exploit the excellent biological properties of the materials while maintaining mechanically viable implants [4, 5].

There is now increasing evidence that surface topography both on the micro and nano-scale are important in determining the cell response to biomaterials [6–11]. A number of studies have indicated that cell activity can be up-regulated through optimization of the surface properties of the substrate. Investigations originally concentrated on surface features of several tens of micrometers in scale, but more recently, evidence suggests that surface topography at a much finer scale may influence the cell response.

Hydroxyapatite (HA) has been used in a number of different forms and several powder processing routes have been investigated in conjunction with HA [12–18]. The application of jet-based suspension processing methods, such as ink-jet printing (IJP) [19, 20] and electrostatic atomization printing (EAP) [21, 22] allows solid freeforming of fine structures of advanced materials. In particular, EAP in the cone-jet mode enables the formation of $<50\ \mu$m size relics of advanced materials, an order of magnitude finer than those produced using IJP, using needles having an internal diameter of $\sim 200\ \mu$m, $\times 4$ coarser than those used in IJP. However, electrostatic atomization or electrospraying has been developed largely by the aerosol industry to process liquids and, only very recently, to process suspensions containing micrometer size particles [21, 22]. In this letter we describe the use of electrospraying to process a suspension contain nano-hydroxyapatite (nHA) particles to deposit droplets, which after spreading on a glass substrate, allows the preparation of relics $<1\ \mu$m in size.

nHA was synthesized by a precipitation reaction between calcium hydroxide ($Ca(OH)_2$) and orthophosphoric acid (H_3PO_4) with a Ca/P ratio of 1.67. Both reagents were AnalaR grade, obtained from BDH, UK. 0.3 M H_3PO_4 solution was added drop wise to 0.5 M $Ca(OH)_2$ solution under continuous stirring at room temperature, while the pH was kept above 10.5 by the addition of ammonia solution. The stirring was maintained for a further 16 h after complete addition of the reactants. The precipitate obtained was aged for a further week and then washed with boiling water. Transmission electron microscopy (TEM, a Jeol 200CX transmission electron microscope at accelerating voltage of 200 keV) of the precipitate revealed that rod-like nHA particles with the size of 50 to 80 nm were obtained and selected area diffraction (SAD) showed a spotted pattern, indicating a polycrystalline material (Fig. 1). The structure of nHA was studied by X-ray diffraction (a Philips PW1730 diffractometer using Cu$K\alpha$ radiation), and the product was found to be phase pure HA.

The nHA particles were suspended in ethanol to give a slurry with a concentration of 3 vol.% nHA.

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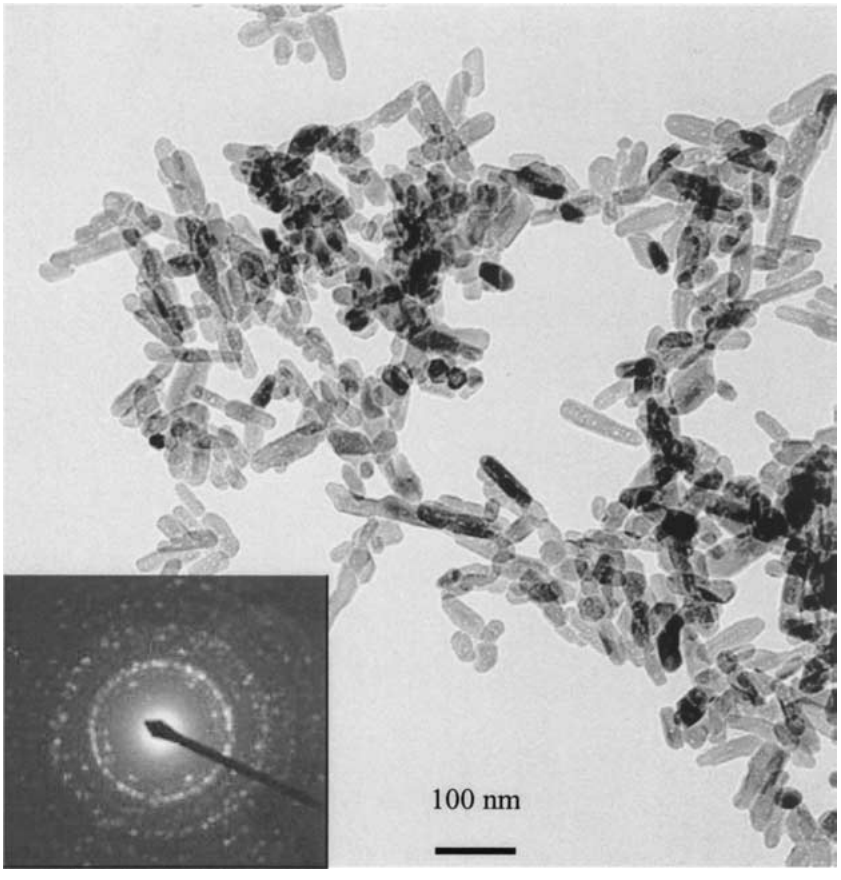

Figure 1 Transmission electron micrograph and selected area diffraction of nHA particles.

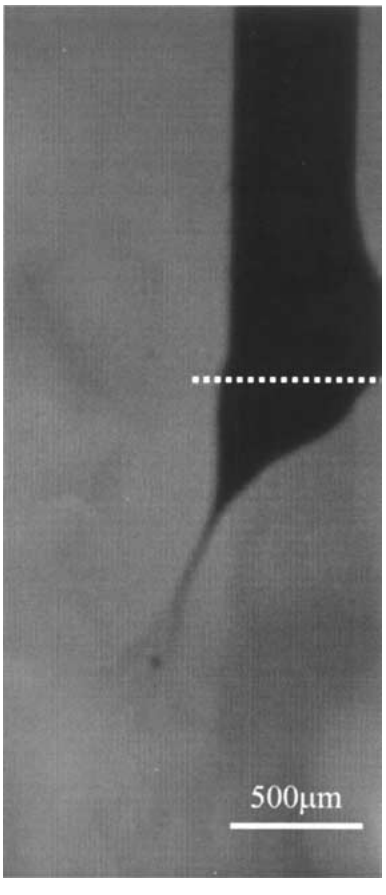

Figure 2 Electrospraying of the nHA suspension as captured by a MotionBlitz high-speed camera (Weinberger AG, Dietikon, Switzerland). The dotted line represents the exit of the needle.

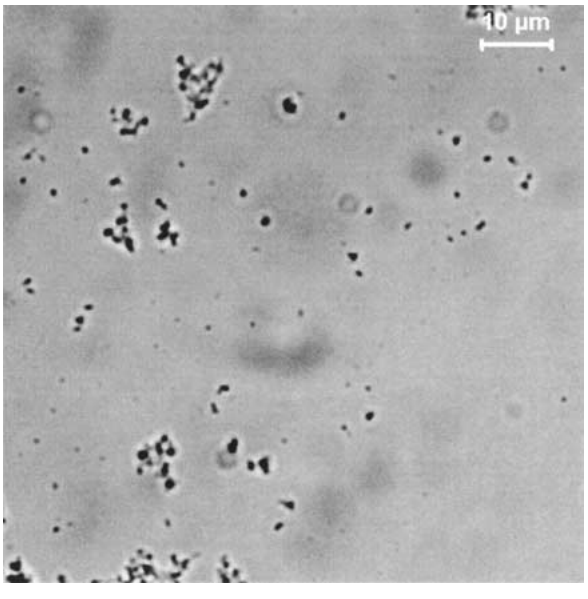

Figure 3 Optical micrograph of electrosprayed nHA relics on a glass slide.

Electrospraying was carried out using the equipment previously reported [21, 22] but with a ring-shaped ground electrode, which produces a spray rather than a focused stream of droplets. In the equipment used, the stainless steel needle had an inner diameter of 200 μm and the ring-shaped ground electrode was held 8 mm below the exit of the needle. Freshly prepared nHA suspension was syringed to the needle at 1.7×10^{-9} m^3 s^{-1}

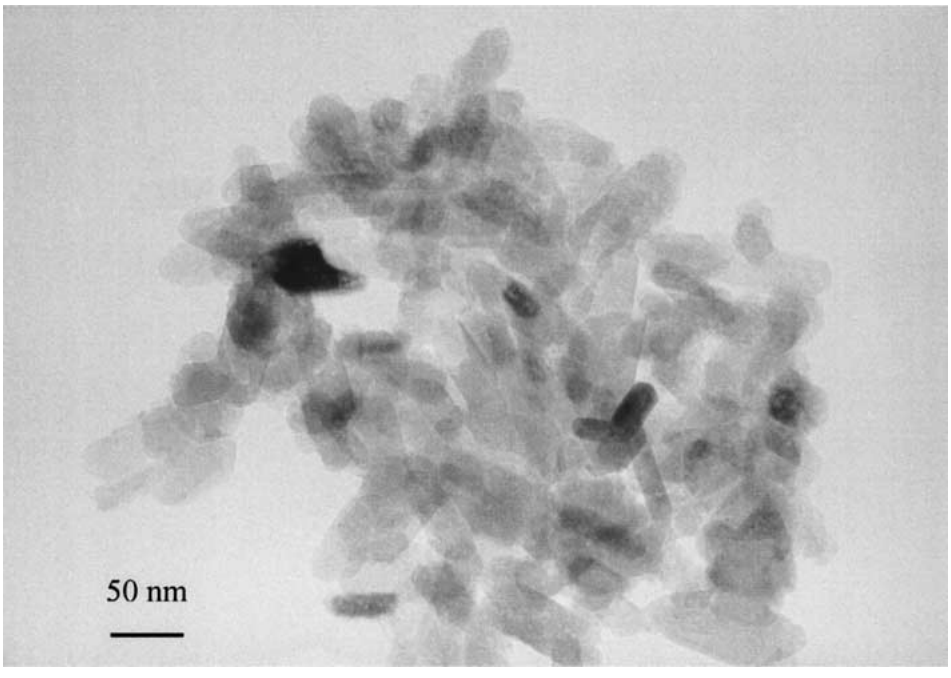

Figure 4 Transmission electron micrograph of typical nHA relic produced by electrospraying.

with the applied voltage set at 6 kV. Under these conditions, with the ammeter reading ∼60 nA, stable electrospraying was achieved, the mode resembling that of the cone-jet (Fig. 2). However, it can also be an electrically forced jet rather than electrohydrodynamic atomization [23] and a full assessment of suspension properties (electrical conductivity, relative permittivity, viscosity, surface tension, density) is necessary to classify the process in greater detail and this is in progress.

nHA droplets were sprayed both on glass (slides) and TEM copper grids (with a carbon support film) for morphological examination. nHA relics on the glass slide were studied under a Leica digital (DC500) optical microscope (DM RXA2). A range of relic sizes, some in the submicrometer range, were found on the glass slide, as shown in Fig. 3. More details of the structure of relics were revealed by TEM, which showed that the nHA relics comprised hexagonal shaped particles of about 50 nm in length (Fig. 4). Therefore, it was possible to produce nHA relics of less than 0.5 μm in size by electrospraying, thus illustrating the significant advantage in processing nano-suspensions using this method.

The nHA relics on the glass substrate were studied by atomic force microscopy (digital instrument,

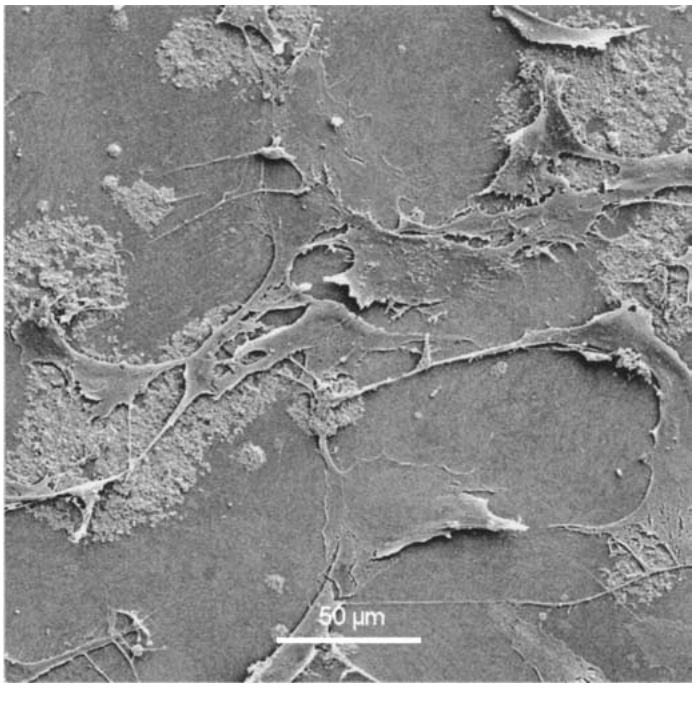

Figure 5 Morphology of HOB cells on nHA relics after 7 days of culture.

nanoscope III) to provide a 3D image with topographical contrast. The equipment was operated in the non-contact mode, where attractive Van der Waals forces acting between the tip and the sample were detected, and topographic images are constructed by scanning the tip above the surface. The results showed that the nHA particle size is less 80 nm, the mean surface roughness is about 33.4 nm.

A biocompatibility assessment was carried out using primary human osteoblast (HOB) cells (Promocell, UK). The glass slides with nHA relics were sterilized by dry heat at 160 °C for 4 h, HOB cells (2×10^4 cells) were then seeded directly on the surface of these substrates and incubated at 37 °C in a humidified air atmosphere of 5% CO_2. The growth of HOB cells on the nHA sprayed glass slides was examined on a regular basis under an optical microscope to monitor any changes in the cultures. After 7 days of incubation, the cultures were fixed, stained with 1% osmium tetroxide and dehydrated in a graduated series of alcohols and finally critical point dried (Polaron E3000 CPD). The sample surface was coated with a thin layer of carbon before it was examined under a scanning electron microscope (Jeol 5800). HOB cells were able to attach to the substrates and generally maintained their osteoblastic morphology. Good cell coverage on the surface was observed with cells having visible filapodia attached to nHA relics (Fig. 5). The result indicated that nHA deposited substrates were able to support the growth of HOB cells.

In summary, biocompatible submicrometer sized relics containing nanosized HA were produced by electrospraying. This procedure offers the potential to create both micro- and nano- scale surface topography and coverage for a favorable cell response.

We are currently exploring the processing of nHA suspensions using different modes of electrospraying and a variety of substrates having different surface porosities. The spreading of the droplets can be controlled even further to prepare even finer relics and, moreover, we are attempting to decrease the needle diameters appreciably, to prepare the finest relics, which may contain only a few nHA particles in them.

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Received 21 August
and accepted 9 September 2003