Calcium Cl/OH-apatite, Cl/OH-apatite/Al$_2$O$_3$ and Ca$_3$(PO$_4$)$_2$ fibre nonwovens: Potential ceramic components for osteosynthesis

Alexander Müller [a,b], Thomas Schleid [c], Michael Doser [d], Nicole Müschenborn [d],
Andrea Tautzenberger [e], Anita Ignatius [e], Bernd Clauß [b], Michael R. Buchmeiser [a,b,∗]

[a] *Institute of Polymer Chemistry, University of Stuttgart, Pfaffenwaldring 55, D-70569 Stuttgart, Germany*
[b] *Institute of Textile Chemistry and Chemical Fibres, Körschtalstraße 26, D-73770 Denkendorf, Germany*
[c] *Institute of Inorganic Chemistry, University of Stuttgart, Pfaffenwaldring 55, D-70569 Stuttgart, Germany*
[d] *Institute of Textile Technology and Process Engineering, Körschtalstraße 26, D-73770 Denkendorf, Germany*
[e] *Institute of Orthopaedic Research and Biomechanics, Helmholtzstraße 14, D-89081 Ulm, Germany*

Abstract

Polycrystalline calcium phosphate ((Cl/OH)Ap = Ca$_5$(PO$_4$)$_3$(OH/Cl); TCP = Ca$_3$(PO$_4$)$_2$) fibres were prepared from aqueous solutions of calcium chloride and phosphoric acid using poly(ethylene oxide) (PEO) as spinning aid. Generation of nonwoven materials was accomplished via rotary jet spinning. Polycrystalline (Cl/OH)Ap fibres 10–25 μm in diameter were obtained with 37% ceramic yield by pyrolysis of the green fibres followed by sintering at 1150 °C in air. X-ray diffraction (XRD) analysis provided evidence for apatite formation starting at 650 °C while (Cl/OH)Ap ceramic fibres were obtained at 1100 °C via transformation through intermediate dicalcium dichloride hydrogen phosphate (Ca$_2$Cl$_2$(HPO$_4$)) and calcium pyrophosphate (Ca$_2$P$_2$O$_7$) phases. A glass-forming Al-based additive was applied to enhance the mechanical properties of the (Cl/OH)Ap ceramic fibres and indeed resulted in the formation of (Cl/OH)Ap/Al$_2$O$_3$ fibres with improved mechanical stability. Finally, TCP, (Cl/OH)Ap and (Cl/OH)Ap/Al$_2$O$_3$ fibres were subjected to seeding with mesenchymal stem cells. Negligible cytotoxicity is observed.

1. Introduction

Calcium phosphate (CaO/P$_2$O$_5$/(H$_2$O), CaP) ceramics show excellent behaviour in terms of biocompatibility, bioactivity and controllable rate of degradation/bone formation as artificial materials for tissue engineering in osteosynthesis. Their mechanical properties, nearly bone-like thermal capacity and controllable porosity complete the application profile of CaP ceramics for bone reconstruction. Calcium orthophosphate implant materials are presently available and used in tailored defect-dependent forms and material shapes, mainly in form of 3D open porous moulded bodies, particulates, cements, coatings and as injectable composites or self-setting cements.[1–3] Despite intensive efforts, it has not yet been possible to develop an artificial CaP implant exhibiting a reversible flexibility while maintaining spongiosa-like structures with a trabeculae scaffold-like natural occurring bone tissue. Such a material would exhibit great potential for occupying bone defects in conjunction with bone cements of higher biodegradation rate or as reinforcing component of CMC-mouldings (CMC = ceramic matrix composites) for osteosynthesis.

One promising approach entails the generation of (Cl/OH)Ap ceramic fibres and their application as open porous, nonwoven materials with reversible deformation and flexible shaping. Such ceramic nonwoven materials would exhibit bio-compatible, bone analogous chemical composition and a trabeculae form as well as open porosity and reversible flexibility. A non-woven ceramic fibre is preferred over a woven one since

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three-dimensional structures can be directly prepared in form of a nonwoven without the need for an additional processing of a single filament ceramic fibre. Up to now only few attempts have been made to develop suitable spinning dopes and fibre spinning processes leading directly to a nonwoven material composed of compact CaP ceramic fibres. Chemically, the majority of CaP bioceramics is based on β-tricalcium phosphate (β-TCP) and hydroxyl apatite ((OH)Ap); their preparation techniques are as manifold as their fields of application.[4–7] Fibrous or needle-like CaP solids are accessible through many general routes of synthesis, e.g., solid state synthesis of apatite with calcium metaphosphate and calcium oxide as well as calcium hydroxide or calcium carbonate as shown by Ota et al.[8] Hideo and co-inventors claimed a fibre production process for CaP ceramic fibres through melt-spinning at temperatures above 1600 °C.[9] However, due to melt preparation, the apatite phases lack any hydroxyl substitution in the apatite crystal structure and consequently any biological activity.[10] Joo et al. developed a water based sol–gel system for green fibre fabrication via electrospinning leading to (OH)Ap nanofibre matrices consisting of hollow, compact or core–shell structured CaP/polymer or CaP fibres. The thermal conversion of these materials led to CaP ceramic fibre mats. Despite their excellent properties as an implant material, the spinning dopes are limited in terms of ageing and the process of electrospinning as such is restricted to small diameter fibres. Unfortunately, inorganic fibres with such small diameters are still medically monitored in terms of carcinogenic properties. Fuji and co-inventors described a slurry-based process to generate cotton-like materials via a cast-process.[11] These most promising materials, however, lack structural homogeneity after sintering to ceramic CaP fibres. Another attempt to fabricate suitable fibrous materials for bone tissue engineering was carried out by Blenk.[12] Coating of polyester fibres with a CaP-slurry led to hollow TCP fibres after pyrolysis and sintering. Despite all attempts to fabricate nonwoven CaP ceramic fibre materials an overall straightforward, cost-efficient way of fabrication such ceramic nonwoven implants is currently not at hand.

The aim of the present work was both to develop spinning dopes with defined properties in terms of rheology and stability and to reveal an expedient way for the direct fabrication of green-fibres (unburned precursor fibres) and their thermal conversion into nonwoven (Cl/OH)Ap ceramic fibres. Finally, preliminary cell toxicological experiments were to be carried out.

2. Materials and methods

2.1. Spinning dope

The CaP precursor spinning dope was prepared by using calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) and phosphoric acid (H_3PO_4, 85 wt.%) as raw materials. To obtain a solution suitable for fibre spinning, poly(ethylene oxide) (PEO, Sigma Aldrich, >99%) was used as spinning aid and dissolved in a mixture of 60 vol.% double distilled H_2O and 40 vol.% ethanol (EtOH, Th.

Geyer, p.a.). The (Cl/OH)Ap precursor solution with a Ca:P molar ratio of 5:3 was prepared by dissolving $CaCl_2 \cdot 2H_2O$ (Sigma Aldrich, 99 wt.%) in the transparent polymer solution. The Ca:P molar ratio can also be varied to obtain other CaP ceramics, e.g., tricalcium phosphate (TCP, Ca:P ratio 3:2). After addition of orthophosphoric acid the solution was stirred at room temperature for 30 min. The pH of the dopes was adjusted to values below pH = 3, best to pH = 0–1 with 1 M hydrochloric acid to ensure highest solubility of the raw materials (as well as stable intermediate species) in aqueous/alcoholic solution and subsequently concentrated in a rotary evaporator to adjust the required viscosity for fibre spinning. The adjustment of viscosity and the characterization of the flow properties were realized by rheological measurements. These measurements were carried out using a Rheometrics dynamic stress rheometer DSR 500 (Peltier element, plate-plate geometry, $d = 40$ mm, gap = 1 mm). To ensure viscoelasticity and suitable elasticity/viscosity characteristics the dynamic frequency sweep mode was applied and spinning dopes were measured in the frequency range from $\omega = 0.1$ rad/s to 550 rad/s in the region of linear-viscoelasticity.

2.2. Spinning procedure

Fibres were hand drawn for first thermal conversion experiments while nonwoven materials were generated as open-porous materials by rotation jet spinning. For hand drawn fibres, the viscous CaP precursor solution was cast between two metal bars and withdrawn in length up to 15 cm. In order to obtain defined fibre diameters and to provide direct access to green fibre nonwoven materials the spinning dopes were spun via the rotary jet spinning process (RJS). Rotary jet spinning was performed on a modified Super Breeze cotton candy machine. Modifications that were carried out entailed the sealing of gaps to protect against electricity, substitution of the stenopaeic blend by a blend with designed orifice geometries and addition of rotation speed regulation as well as a of a hot air fanner. Degassing of the spinning dopes prior to the spinning process was not required due to the relatively low viscosities. The spinning dopes (zero shear viscosity η_0 up to 30 Pa s, surface tension $\gamma = 44$ mN/m and density $\rho = 3$ g/cm^3) with a polymer content of at least 20 wt.% of polymer were placed in the reservoir of the RJS spinning head ($V = 450$ ml, external diameter $r_{ext} = 7.2$ cm). Continuous refilling allowed for maintaining both the hydrostatic pressure and continuous flow during spinning. The principles of the RJS process for nanofibre materials were briefly investigated by Badossamay et al.[13] They can generally be subdivided into (i) jet-initiation, (ii) jet-extension and (iii) solidification and shrinking of the polymer jet. Such a horizontal, high-speed rotary extrusion ($U = 2300$ rpm) of the spinning dopes through pinholes with selectable orifice geometry ($L:D = 4.5$) into a heated interior ($T = 70$ °C) led to the green fibre material as a nonwoven with fibre diameters between 10 and 25 μm. This green fibre nonwoven was collected on a PTFE fabric placed in a 25 cm distance from the orifices. The resulting battings of green fibres were then boxed in and stored or directly subjected to further drying ($t = 30$ min, $T = 100$ °C) to remove residual water.