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Electrochemical microelectrodes for improved spatial and temporal characterization of aqueous environments around calcium phosphate cements

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ABSTRACT

Calcium phosphate compounds can potentially influence cellular fate through ionic substitutions. However, to be able to turn such solution-mediated processes into successful directors of cellular response, a perfect understanding of the material-induced chemical reactions in situ is required. We therefore report on the application of home-made electrochemical microelectrodes, tested as pH and chloride sensors, for precise spatial and temporal characterization of different aqueous environments around calcium phosphate-based biomaterials prepared from α-tricalcium phosphate using clinically relevant liquid to powder ratios. The small size of the electrodes allowed for online measurements in traditionally inaccessible in vitro environments, such as the immediate material–liquid interface and the interior of curing bone cement. The kinetic data obtained has been compared to theoretical sorption models, confirming that the proposed setup can provide key information for improved understanding of the biochemical environment imposed by chemically reactive biomaterials.

1. Introduction

Enabling tools that provide accurate spatial and temporal information on cellular fate and/or material performance find numerous applications in biomaterials and tissue engineering research [1]. Ion and pH sensors are examples of enabling tools that can reveal information on both tissue development and material activity. For example, general cellular respiration tends to provoke acidification of the extracellular environment [2], and in bone tissue engineering applications specific cellular activity may further influence the extracellular pH, as well as the ionic environment with respect to calcium and phosphate [3–5]. In parallel, many scaffold biomaterials used in bone tissue engineering are based on calcium phosphate compounds [6] which often undergo ion-exchange processes when immersed in aqueous environments [7,8]. Such solution-mediated reactions may simultaneously influence pH and the concentration of many extracellular ions, which in turn can affect cellular behaviour both positively and negatively [9–12].

To control or turn such material-induced ionic interactions into a powerful tool that can promote or suppress certain cellular activity, one first has to understand the nature of the reactions, and determine their magnitude in biologically relevant volumes and environments. Of particular interest are environments created at the immediate interface between biomaterials and biological substances, as well as in the interior of scaffolds. Detailed characterizations of such environments are still scarce [13–14], but recent efforts to develop biocompatible optical microparticle sensors may improve our understanding of the instantaneous composition of local microenvironments at the cell–material interface [15,16]. A different method to approach local environments at the material interface includes the use of miniaturized electrochemical sensors. Electrochemical sensors do not only have a long history of applications in complex chemical environments, but are also relatively easy to modify for the detection of many different analytes and in different environments, including opaque ones where optical sensors may not be used.

In this study we have focused on the use of electrochemical sensors for spatial and temporal evaluation of small-volume environments created around ion-reactive calcium phosphate cements. For this purpose, we present how miniaturized electrodes were prepared from iridium oxide (IrO_2) or silver/silver chloride (Ag/AgCl) to obtain sensors for pH or chloride ions, respectively, and how those sensors were subsequently used in standard in vitro environments containing ion-reactive biomaterials. As a model biomaterial we used α-tricalcium phosphate (α-TCP), which

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undergoes a hydrolysis reaction to yield calcium-deficient hydroxyapatite (CDHA) when mixed with water.

Both α-TCP and CDHA, either in isolation or in different combinations with other calcium phosphate compounds, have been explored as scaffold candidate materials in bone tissue engineering and as drug delivery vehicles [12,17,18]. In these applications the reactive biomaterial inevitably comes into contact with living tissue and/or biomolecules susceptible to changes in the aqueous chemical environment. Therefore, a careful characterization of how calcium phosphate cements influence their adjacent aqueous environments during their setting reaction is highly desirable. To that end, the described microelectrodes were positioned either directly in the cement paste upon preparation or were placed at the immediate interface between the biomaterial and the surrounding liquid. The kinetic data obtained is compared to theoretical sorption models in order to better understand the underlying mechanisms that determine the chemical environment around α-TCP and CDHA.

2. Materials and methods

2.1. Fabrication of the microelectrode body

Two different sensors were used in this work: (i) Ag/AgCl electrodes for detection of chloride ion activity and (ii) pH electrodes of electrodeposited anodic iridium oxide film (AIROF) on gold. While their chemical structures are completely different, they shared a similar physical electrode design (Fig. 1a), previously described by Bitziou et al. [19]. Basically, the electrodes were prepared from Teflon-insulated Ag or Au wires (Advent Research Materials Ltd., AG549511 and AU518710: diameter 0.2 mm and 0.75 μm, respectively), which were threaded through a syringe needle (BD Microlance 3, 0.9 × 25 mm). Epoxy resin (Robnor, PX771C) was used to backfill the needle to fix and isolate the metal wires within the needle body. Upon resin curing, the sharp end of the needle was cut flat using a rotating diamond saw blade and polished in four subsequent steps: first with silicon carbide grinding paper (Buehler, Grit P400/800) and then with slurries of alumina microparticles (Buehler; 1.0, 0.3 and 0.05 μm, respectively). With this method, needles that contained one or multiple flat and circular Ag or Au microelectrodes were successfully fabricated (Fig. 1b and c), and which could be easily incorporated into the surface of mouldable biomaterials (Fig. 1d and e). However, before incorporation into any biomaterial, the surface of the metal electrodes was chemically modified, as described below, with iridium oxide or AgCl in order to convert them to pH or chloride sensors, respectively.

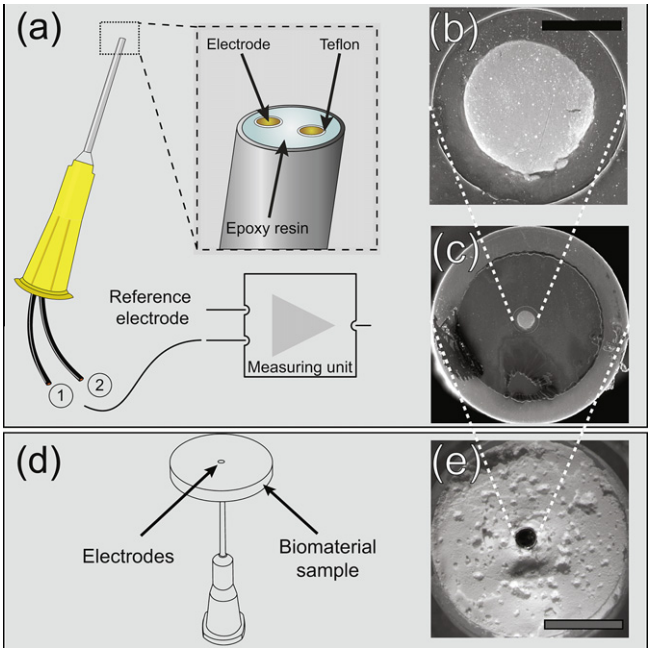

Fig. 1. (a) Schematic view of the sensor body (not drawn to scale), here drawn with two microelectrodes incorporated at its tip. (b, c) Scanning electron microscope images of the sensor needle tip containing one bare Au microelectrode. Black scale bar = 50 μm. (d) Schematic view of the setup for electrochemical measurements at the biomaterial–liquid interface. (e) Incorporation of the sensor body in CDHA for measurements at the material/liquid interface. Grey scale bar = 3.6 mm.

2.2. Ag/AgCl microelectrodes

After preparation of Ag microelectrodes according to Section 2.1, electrochemical deposition of AgCl onto the Ag electrodes was performed electrochemically. For that purpose, the Ag electrode was connected to the anode of a 4.5 V battery and immersed in a solution of 0.1 M HCl. A platinum wire (Advent Research Materials Ltd., PT541507), in series with a resistor to provide a current density of about 0.4 mA cm^{-2}, was connected to the cathode of the battery for 40 min [20]. Before the obtained Ag/AgCl electrode was used, it was left in distilled H_2O overnight.

2.3. pH microelectrodes based on IrO_2 deposited onto Au

Au microelectrodes were prepared according to Section 2.1 and further cleaned by potential cycling in 0.5 M H_2SO_4 (0–1.4 V, scan rate of 50 mV s^{-1}), using a platinum counter electrode (BASi, MW-1032) and a Ag/AgCl double junction reference electrode (Thermo Scientific, Orion 900200). Iridium oxide films were then formed on the Au microelectrodes by anodic electrodeposition using an alkaline iridium tetrachloride solution [19,21], which was prepared by dissolving 0.15 g of iridium (IV) chloride hydrate ($IrCl_4 \cdot H_2O$, Sigma 516996) in 100 ml of milliQ water. After 30 min of stirring, 1 ml of aqueous hydrogen peroxide (H_2O_2 33% w/v, Panreac 211077 1214) was added, followed by 0.5 g of oxalic acid ($(COOH)_2 \cdot 2H_2O$, Sigma 247537) after another 30 min, in order to avoid spontaneous precipitation of IrO_2. Once again, the solution was stirred for 30 min. Finally, about 5 g of anhydrous potassium carbonate (Sigma, 590681) was gradually added to adjust the pH of the solution to 10.5. The resulting solution, having a pale green-yellowish colour, was left standing for 2 days at room temperature until the colour changed to blue. From then on, the solution was stored at a temperature of 4 °C, and could be used for several months to successfully add AIROFs to Au microelectrodes by electrodeposition. For the latter process, a potentiostat (CH Instruments CHI1232) and the same reference and counter electrodes as indicated above were used to apply a constant potential (0.65 V) for 3 min while using the Au microelectrode as the working electrode. After deposition, the coated microelectrodes were washed and left in water for hydration of the AIROF for at least 2 days prior to use as pH sensors.

2.4. Potentiometric measurements

Real-time potentiometric measurements between the fabricated microelectrodes and a commercial reference electrode were performed with an electronic unit that was fabricated in-house, based on an instrumentation amplifier (Ultra Low Input Bias Current INA116, Burr–Brown). The output signal, i.e. the voltage difference between the indicator and the reference electrode, was read with a DAQ-board (National Instruments, USB6009) that was connected via USB to a PC. The acquired data was logged with home-written software (LabView 8.2). This setup allowed for simulta-