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Preparation of SBF with different HCO_3^- content and its influence on the composition of biomimetic apatites

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Abstract

The bioactivity of bone and dental implant materials is usually tested in vitro using simulated body fluid (SBF). The composition of common SBF differs from that of blood plasma in that it has a higher Cl^- and a lower HCO_3^- concentration, which affects the composition of in vitro formed bone-like apatite. Five different SBFs with a composition of 142 Na^+, 5 K^+, 2.5 Ca^{2+}, 1 Mg^{2+}, 1 SO_4^{2-}, 1 HPO_4^{2-}, and 136 ($Cl^- + HCO_3^-$) mmol/l were prepared with HCO_3^- concentrations ranging from 5 to 27 mmol/l. The SBF solutions were prepared by mixing stable concentrated solutions, which increase the reproducibility of in vitro tests due to negligible changes of pH during preparation. The high stability of thus prepared SBF enables the evaluation of hydroxyapatite formation on the surface of bioactive materials without the negative effect of spontaneous precipitation. Furthermore, the use of concentrated solutions offers a facile way to prepare SBF with different ionic contents and thus modify the composition of Ca–P layers precipitated on the surface of the bioactive materials exposed to the SBF solutions. The SBF solutions were shown to be supersaturated with respect to slightly carbonated apatite. The Fourier transform infrared (FT-IR), Raman and X-ray analyses of the precipitated layers indicate that the HCO_3^- content in SBF influences the composition and structure of the calcium phosphates obtained. It can be supposed that as long as the HCO_3^- concentration in the testing solutions is lower than 20 mmol/l, only B-type HCA precipitates. At higher HCO_3^- concentrations, it can be assumed that A-type HCA forms as well considering FT-IR, Raman and X-ray measurements.

Keywords: Simulated body fluid; In vitro bioactivity test; Hydroxyapatite; Bioactive materials

1. Introduction

Artificial materials considered to be suitable for applications as implant materials are commonly tested in vivo and by in vitro methods in media simulating body fluid. These tests are focused on the examination of physical, chemical and mechanical properties of materials and thus provide the basic information needed to judge their suitability for clinical use in the human body [1,2].

During in vitro bioactivity tests, the material is exposed to an aqueous solution simulating the inorganic part of blood plasma in the presence or absence of cell cultures, and the interactions of the surface with the solution are examined. Both ground and compact samples of implant materials are tested, and changes in the concentration of individual components in the model solutions as well as changes in the surface of the immersed material samples are studied [1,3–5]. As the conditions of the in vitro experiments described in the literature have not yet been standardized, it is always necessary to precisely specify the temperature, composition and pH of the leaching solution, the time and nature of exposure (static or dynamic conditions) and the ratio of the material's surface area to the volume of the leaching solution (S/V), etc. In vitro test results could be significantly influenced by changing just one of the conditions described above [6–8].

Electrolyte solutions, referred to as simulated body fluid (SBF), are the most favored model solutions described in

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Table 1
Comparison of the ionic concentrations in blood plasma and in other physiological solutions [mmol/l]

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
Blood plasma	142.0	3.6–5.5	1.0	2.1–2.6	95.0–107.0	27.0	0.65–1.45	1.0
SBF	142.0	6.5	1.5	2.5	148.0	4.2	1.0	0
Ringer's sol.	39.1	1.4	0	0.4	40.7	0.6	0	0
HBSS	141.7	5.7	0.8	1.7	145.6	4.2	0.7	0.8
199-medium	0	0	0	27.8	0	9.8	27.1	0

the literature for the assessment of materials' bioactivity by evaluating their ability to induce apatite formation on their surface [2]. However, SBF simulates just the inorganic part of human blood plasma and does not contain proteins, glucose, vitamins, hormones, etc. The amount of some ions in human blood plasma (i.e. K^+, Ca^{2+}, Cl^- and HPO_4^{2-}) can vary depending on the person's sex, age and nutrition. A comparison of the concentrations of ions (mmol/l) in human plasma and in SBF is given in Table 1. Further testing solutions mentioned in the literature including Ringer's solution, the "HBSS" model solution and the "199 medium" model solution are also summarized in Table 1. "HBSS" contains 1 g/l d-glucose and "199-medium" contains 0.4 g $Ca_3(C_6H_5O_7) \cdot 4H_2O$, 0.885 g alkaline phosphatase, 0.03 g thymol blue and 0.05 g phenol red per litre of solution [8,9].

The interest in using SBF for in vitro tests [9,10] as well as for preparation of biomimetic apatite coatings has greatly increased within the last 5 years [11–15]. Thus, several SBF solutions such as revised (r-SBF) and modified (m-SBF) with ionic concentrations closer or equal to that of human blood plasma have been prepared [16]. However, the preparation of SBF has to be carried out under well controlled conditions to avoid coagulation and precipitation that could influence the in vitro test results.

The aim of the present study was to develop a new method for SBF preparation that increases the reproducibility of the SBF test procedure and thus improves the reproducibility of in vitro tests. Furthermore, the influence of the HCO_3^- content on the apatite formation and supersaturation with respect to different calcium phosphates and calcite was examined.

2. Materials and methods

2.1. Preparation of SBF

Five SBF solutions (SBF5, SBF10, SBF15, SBF20, SBF27) with a HCO_3^- content ranging from 5 to 27 mmol/l and a constant ($Cl^- + HCO_3^-$) content of 136 mmol/l were prepared by pipetting calculated amounts of concentrated solutions of KCl (59.64 g/l), NaCl (116.88 g/l), $NaHCO_3$ (45.37 g/l), $MgSO_4 \cdot 7H_2O$ (49.30 g/l), $CaCl_2$ (prepared from $Ca(OH)_2$, see below), TRIS (tris-hydroxymethyl aminomethan; 121.16 g/l), NaN_3 (100 g/l) and KH_2PO_4 (27.22 g/l) into double distilled water to prevent precipitation of homogeneously nucleated calcium phosphates or other phases and to minimize changes in pH during preparation.

$Ca(OH)_2$ powder was suspended in 100 ml double distilled H_2O and dissolved by adding of a calculated amount of HCl (e.g. 17.16 ml of 36% HCl or 17.76 ml of 35% HCl). The clear solution of $CaCl_2$ was quantitatively transfused into a 1000 ml flask and filled up with double distilled H_2O.

The 121.16 g of TRIS was dissolved in 650 ml double distilled H_2O. During stirring, the pH was adjusted to 7.6–7.7 at 25 °C, which is equal to a pH of 7.3–7.4 at 37 °C, by adding concentrated HCl. The clear solution of TRIS and HCl was quantitatively transfused into a 1000 ml flask and filled up with double distilled H_2O. All salt solutions were stored in polyethylene (PE) bottles in a refrigerator at a temperature of 5 °C. Under these conditions the solutions remained stable over a period of 12 months.

The pipette volumes of concentrated salt solutions to prepare 1 l SBF are listed in Table 2. The solutions were pipetted into 700 ml double distilled water in the sequence KCl, NaCl, $NaHCO_3$, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, (TRIS + HCl), NaN_3 and KH_2PO_4 to prevent precipitation. The pH of human blood plasma ranges from 7.3 to 7.4 at 37 °C. Since the pH of SBF depends on temperature and linearly decreases with increasing temperature by 2.813×10^{-3}/°C, the pH of SBF prepared at room temperature (21 °C) has to be adjusted between 7.75 and 7.85 in order to obtain 7.3 and 7.4 at 37 °C. The compositions of model solutions are given in Table 3. The microbiological analysis of SBF indicated the necessity of NaN_3 addition to inhibit the growth of bacteria. In SBF without NaN_3 a massive increase of cells (10^4–10^5/ml) in the form of *coliform bacterium*, *bacterium bacillus* and *Pseudomonas* was observed after cultivation in culture media at 37 °C. These anaerobic bacteria consume phosphorus and thus reduce

Table 2
Pipette volume of concentrated salt solution to prepare 1 l SBF [ml]

Solution	SBF5	SBF10	SBF15	SBF20	SBF27
KCl	5	5	5	5	5
NaCl	60	59	56	53.5	50
$NaHCO_3$	10	18	27.8	37	50
$MgSO_4 \cdot 7H_2O$	5	5	5	5	5
$CaCl_2$	25	25	25	25	25
TRIS + HCl	50	50	50	50	50
NaN_3	10	10	10	10	10
KH_2PO_4	5	5	5	5	5

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