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Using a synthetic body fluid (SBF) solution of 27 mM HCO_3^- to make bone substitutes more osteointegrative ☆

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Abstract

A Tris–HCl-buffered synthetic body fluid (SBF) solution, mimicking the human blood plasma, with the following ion concentrations of 27 mM HCO_3^-, 2.5 mM Ca^{2+}, 1.0 mM HPO_4^{2-}, 142 mM Na^+, 125 mM Cl^-, 5 mM K^+, 1.5 mM Mg^{2+}, and 0.5 mM SO_4^{2-} was used as an aqueous medium to process a number of bone substitute materials under the so-called biomimetic conditions of 37 °C and pH 7.4. This solution was named as Tris–SBF-27 mM. Firstly, collagen sponges were soaked in Tris–SBF-27 mM solution at 37 °C and were found to be fully covered with nanoporous apatitic calcium phosphate (Ap-CaP). The composites of collagen–Ap-CaP biomaterials are expected to be used in orthopedic and dental surgery. Secondly, Ap-CaP short whiskers or microrods with a novel nanotexture and surface areas higher than 45 m^2/g were synthesized in Tris–SBF-27 mM solution. Thirdly, calcium sulfate cements doped with $CaHPO_4$ (monetite), were shown to have apatite-inducing ability upon ageing in Tris–SBF-27 mM. $CaHPO_4$ addition in calcium sulfate was found to improve its mechanical strength, measured after cement setting reaction. Pure calcium sulfate cement pellets were not stable in Tris–SBF-27 mM solutions and crumbled into a powder. All the samples were characterized by SEM, XRD, FTIR, surface area and mechanical strength measurements.

Keywords: Bone substitute; Calcium phosphate; Biomaterial; Synthesis; Body fluid; Biomimetic

1. Introduction

The historical development of synthetic or simulated body fluids (SBF, which claim to mimic the acellular human blood plasma) cannot be pictured accurately without mentioning the Ringer's solution of 1880 [1], Earle's balanced salt solution (*EBSS*) of 1943 [2] and Hanks' balanced salt solution (*HBSS*) of 1949 [3]. SBF solutions developed by T. Kokubo between 1990 [4] and 2006 [5] may be considered as close relatives of EBSS and HBSS.

The ion concentrations of acellular human blood plasma and some physiological solutions are compared in Table 1. HBSS and EBSS solutions, on the other hand, are commercially available today as either supplemented with glucose or with amino acids and vitamins, and are commonly used in tissue engineering or cell culture studies. Solutions such as HBSS and EBSS do help to maintain intra- and extracellular osmotic balance, provide cells with water and certain bulk inorganic ions essential for normal cell metabolism, and provide a buffering system to maintain the medium within the physiological pH range (7.2–7.6) [6].

The HCO_3^- concentration of the EBSS solution (i.e., 26.2 mM) resembles that of human plasma (27 mM). On the other hand, the carbonate ion concentration of the HBSS solution is much lower than that (i.e., 4.2 mM). The SBF (*simulated body fluid*) solution resembled to a Tris–HCl-buffered (at pH 7.4 and 37 °C) HBSS solution whose Ca/P molar ratio was adjusted to 2.50, and whose Mg^{2+} concentration was increased from 0.81 (HBSS) to 1.5 mM [4]. The original HBSS solution had a Ca/P molar ratio of 1.62, whereas the same ratio in EBSS was 1.80 [7,8].

☆ Notes: Certain commercial equipment, instruments or materials are identified in this paper to foster understanding. Such identification does not imply recommendation or endorsement by the author, nor does it imply that the equipment or materials identified are necessarily the best available for the purpose.

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Table 1
Ion concentrations of human plasma and synthetic solutions, mM

	Human plasma	Ringer [1]	EBSS [2]	HBSS [3]	*Kokubo*–SBF [4]	*Tas*–SBF [13,14]
Na^+	142	130	143.6	138	142	142
K^+	5	4	5.37	6.14	5	5
Ca^{2+}	2.5	1.4	1.8	1.26	2.5	2.5
Mg^{2+}	1.5		0.81	0.81	1.5	1.5
Cl^-	103	109	125.3	144.8	147.8	125
HCO_3^-	27		26.2	4.2	4.2	27
HPO_4^{2-}	1		1	0.78	1	1
SO_4^{2-}	0.5		0.81	0.81	0.5	0.5
Ca/P	2.5		1.8	1.62	2.5	2.5
Buffer					*Tris*	*Tris*
pH	7.4	6.5	7.2–7.6	6.7–6.9	7.4	7.4

Is it possible to convert an HBSS solution of 4.2 mM HCO_3^- into an SBF of 4.2 mM HCO_3^-? As shown in Table 1, HBSS has an HPO_4^{2-} concentration of 0.78 mM, and in order to raise this value to 1 mM, one simply needs to add 31.2 mg of anhydrous Na_2HPO_4 to 1 L of a commercially available HBSS solution. By this addition of Na_2HPO_4, the Na^+ concentration of the HBSS solution would increase to 138.44 (from 138 mM). In order to raise the Ca/P molar ratio of the above solution to 2.50, one must add 182.3 mg of $CaCl_2{\cdot}2H_2O$ into this solution. After this addition of Ca-chloride dihydrate, the new solution will have a Ca^{2+} concentration of 2.5 mM (therefore, a Ca/P ratio of 2.50) and Cl^- concentration of 147.28 mM. A small aliquot of 1 M HCl solution can be added (before the introduction of $CaCl_2{\cdot}2H_2O$) to prevent premature CaP precipitation in the solution. Finally, in order to increase the Mg^{2+} concentration of 1 L of the above solution to 1.5 mM, one must add and dissolve 140.3 mg $MgCl_2{\cdot}6H_2O$. This addition will also raise its Cl^- concentration to 147.8 mM. All of these additions do not alter the HCO_3^- concentration, and it will remain constant at 4.2 mM. Adjusting the pH of this solution can easily be achieved by using the Tris (*tris-hydroxymethyl-aminomethane*)–HCl couple. The original SBF solution was, therefore, unable to mimic the human blood plasma in terms of one of its most important ions, namely HCO_3^- [4].

The reader is hereby advised to refer to an experimental comparison of HBSS and the above-mentioned solutions, in terms of their CaP-depositing capacities, being reported in the work of Serro and Saramago [9]. It is a well-known fact that even a pristine HBSS solution is able to deposit CaP on titanium surfaces, but at a much slower pace in comparison to SBF solutions [10–14].

Hepes (*2-(4-(2-hydroxyethyl)-1-piperazinyl)ethane sulphonic acid*), instead of Tris, was also used to stabilize the pH of SBF-like solutions at 7.4 [15]. However, Oyane et al. [15] reported, in comparison to *Tris–HCl-buffered* SBF solutions, that *Hepes–NaOH-buffered* SBF would easily release CO_2 gas from the solution, causing a decrease in its nominal HCO_3^- concentration, and an increase in its pH value, when the storage period was long. Furthermore, Oyane et al. [15] stated that Hepes–NaOH-buffered SBF would not be suitable for long-term use in the biomimetic CaP formation or deposition processes owing to its instability. On the other hand, Ogino et al. [16] have pointed out as early as 1980 that the *in vivo* calcium phosphate formation on glasses can be simulated best in a Tris–HCl-buffered synthetic solution of pH 7.4.

HBSS and EBSS solutions are manufactured on the commercial scale (for instance, by Sigma-Aldrich Corp.) by using the starting inorganic salts of NaCl, KCl, $MgSO_4$, $CaCl_2{\cdot}2H_2O$, KH_2PO_4/NaH_2PO_4 (or Na_2HPO_4) and $NaHCO_3$ [6]. Therefore, in preparing synthetic body fluids one should avoid the use of hygroscopic $CaCl_2$, which rapidly attracts moisture from the atmosphere, in stark contrast to what has always been suggested in the articles of Kokubo [4,5]. SBF solutions must instead be prepared by using $CaCl_2{\cdot}2H_2O$, similar to what the manufacturers of HBSS and EBSS do [6].

This study presents a detailed recipe for preparing Tris–HCl-buffered SBF solutions with an HCO_3^- concentration of 27 mM (just like the human plasma), and exemplifies the use of this solution in synthesizing unique bone substitute (graft) biomaterials, such as biomimetic CaP microrods, collagen–CaP composites, and monetite ($CaHPO_4$)-doped calcium sulfate composites for musculoskeletal repair applications. The Tris–SBF-27 mM solution was originally developed by one of the current authors back in 1999–2000 and used in that period to produce Na, K and Mg-doped apatitic calcium phosphate powders under the biomimetic conditions of 37 °C and pH 7.4 [13,14]. The biomimetic CaP deposition ability of Tris–SBF-27 mM solutions (in direct comparison to Kokubo–SBF solutions) on titanium and its alloys was recently reported elsewhere [17]. On the other hand, the use of the Tris–SBF-27 mM solution of this study has also been tested in improving the biocompatibility of a common polymer such as PTFE (i.e., Teflon®) by Grondahl et al. [18].

2. Experimental

2.1. Preparation of synthetic body fluid (SBF)

The following reagent-grade chemicals were used in preparing Tris–HCl-buffered SBF solutions of 27 mM HCO_3^- in deionized water [13,14]:

(1) sodium chloride (NaCl),
(2) potassium chloride (KCl),
(3) sodium hydrogen carbonate ($NaHCO_3$),
(4) magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$),
(5) sodium sulphate (Na_2SO_4),
(6) calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$),
(7) di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$),
(8) Tris ($(CH_2OH)_3CNH_2$),
(9) 1 M HCl solution.

It is extremely important not to use KCl and $K_2HPO_4{\cdot}3H_2O$ at the same time during the preparation of Tris–HCl-buffered SBF, since this will only lead to an SBF solution with low HCO_3^- concentration (i.e., 4.2 mM). The use of di-sodium hydrogen phosphate in place of di-potassium hydrogen phosphate ensures the attainment of 27 mM HCO_3^- in a Tris–HCl-buffered SBF solution.