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Review

The use of physiological solutions or media in calcium phosphate synthesis and processing

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ABSTRACT

This review examined the literature to spot uses, if any, of physiological solutions/media for the in situ synthesis of calcium phosphates (CaP) under processing conditions (i.e. temperature, pH, concentration of inorganic ions present in media) mimicking those prevalent in the human hard tissue environments. There happens to be a variety of aqueous solutions or media developed for different purposes; sometimes they have been named as physiological saline, isotonic solution, cell culture solution, metastable CaP solution, supersaturated calcification solution, simulated body fluid or even dialysate solution (for dialysis patients). Most of the time such solutions were not used as the aqueous medium to perform the biomimetic synthesis of calcium phosphates, and their use was usually limited to the in vitro testing of synthetic biomaterials. This review illustrates that only a limited number of research studies used physiological solutions or media such as Earle's balanced salt solution, Bachra et al. solutions or Tris-buffered simulated body fluid solution containing 27 mM HCO_3^- for synthesizing CaP, and these studies have consistently reported the formation of X-ray-amorphous CaP nanopowders instead of Ap-CaP or stoichiometric hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) at 37 °C and pH 7.4. By relying on the published articles, this review highlights the significance of the use of aqueous solutions containing 0.8–1.5 mM Mg^{2+}, 22–27 mM HCO_3^-, 142–145 mM Na^+, 5–5.8 mM K^+, 103–133 mM Cl^-, 1.8–3.75 mM Ca^{2+}, and 0.8–1.67 mM HPO_4^{2-}, which essentially mimic the composition and the overall ionic strength of the human extracellular fluid (ECF), in forming the nanospheres of X-ray-amorphous CaP.

1. Extracellular fluid (ECF) of the human body

Water, being vital for all forms of life, covers approximately 70% of the earth's surface, but only 2.5% of this vast amount of water is fresh water. 98.8% of the global fresh water reservoir is contained in ice and groundwater [1]. Water is the main component of biomineralization media in which almost all biological reactions leading to hard tissue formation or ossification takes place [2–4]. Although fresh water is essential to animals and plants, the physiological fluids of animals, which take part in biomineralization events, are not high-purity water. Water in its purest form is an abstraction and is hard to find in earth's aquifers. In laboratory experiments and in most industrial-scale applications, researchers and technicians use distilled, deionized or critical laboratory reagent grade (CLRW) water. According to the definition of Barskov [5], biological mineralization is the coevolution of the biological and mineral worlds. Biological molecules resulting from the normal or pathological metabolism of organisms and the inorganic ions present in their operational environment forming the mineral world interact to create the mineralized tissues of humans, animals and plants [5]. The inorganic ions, such as Ca^{2+}, Mg^{2+}, Na^+, K^+, HPO_4^{2-} (monohydrogen phosphate), HCO_3^- (bicarbonate), Cl^- and SO_4^{2-}, present in biological fluids and cells play a crucial role in the formation of ion-substituted calcium phosphates of hard tissues at the nanoscale. As such, nanotechnology has been with us in its most sophisticated forms (e.g. in the tissues of living creatures) for billions of years much before the invention of this word in 1974 [6].

Physiological fluids of humans can be viewed in two major compartments: intracellular fluid (ICF, with a volume of 27 l for a 70 kg person) and extracellular fluid (ECF, 13 l). Extracellular fluid is then divided into two sub-compartments, interstitial fluid (ISF, 9.5 l) and the liquid component of blood (plasma, 3.5 l for a 70 kg person). ECF represents the fluid outside cells whereas ICF is the fluid within cells. ISF is the tissue fluid found between cells. Plasma contains significantly more protein than does ISF, and the plasma proteins are the only constituents of plasma that do not cross into ISF [7]. There is a striking difference between the compositions of intracellular and extracellular fluids, as shown in Table 1 [8]. The composition of blood plasma is similar to that of ECF [9]. In brief, the plasma membrane of cells is freely permeable to water and

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Table 1
Electrolyte composition of ICF and ECF compartments (in mM).

	Na^+	K^+	Mg^{2+}	Ca^{2+}	HPO_4^{2-}	HCO_3^-	Cl^-	SO_4^{2-}
ICF	10	140	29	0.01	37	10	4	1
ECF	142	5	1.5	2.5	1	27	103	0.5

impermeant to ions other than through ion channels. For instance, NaK-ATPase (the enzyme that splits adenosine triphosphate) is an ion channel, which actively moves K^+ into the cell and removes Na^+ out to maintain the ionic concentrations shown in Table 1 within narrow limits [8].

There is a significant difference between the Ca^{2+} concentrations of intracellular (0.01 mM) and extracellular fluids (2.5 mM). The calcium ion is, pharmacologically, one of the most disruptive substances for normal cell function, with the intracellular concentration of calcium ions carefully regulated [10] and the bones serving as the major storage sites for excess calcium. Based on the accurate elemental analysis of bone and tooth specimens recently given by Castro [11], human bones contain 24.5 wt.% Ca, 11.5% P, 5.8% CO_3, 0.7% Na, 0.55% Mg and 0.03% K, besides a number of trace elements at the ppm level, including Zn, Fe, Sr, Pb, Ba and Cu. Carbonate, sodium, magnesium and potassium ions are the major dopants of apatitic calcium phosphate (Ap-CaP) bone mineral, and the same ions are also present in ECF. The carbonated, ion-substituted, non-stoichiometric and cryptocrystalline Ap-CaP inorganic phase (i.e. biological apatite) of bones in fact stores 99 wt.% of the body's calcium, 80 wt.% of its phosphorus and 50 wt.% of its magnesium [12–14]. In other words, as a toxic ion that needs to be removed from most cells, calcium accumulates extracellularly and the occurrence of calcium deposits may therefore represent a form of detoxification [10,15]. Simkiss [15] briefly described biomineralization as a cellular detoxification mechanism. This review searches the previous literature for solutions mimicking extracellular fluid and their use in synthesizing calcium phosphates.

2. Biomimetic synthesis

The phrase "biomimetic synthesis", according to the Web of Knowledge[SM] database, was first used in 1972 to describe specific synthesis protocols for nicotine [16], macralstonine [17] and villalstonine [18]. Biomimetic synthesis was, therefore, not historically coined for the production of synthetic biomaterials of tissue engineering. Tabushi and Imuta [19] mentioned the biomimetic synthesis of nucleotide phosphates in 1982. Nucleotides are biological molecules that form the building blocks of the nucleic acids DNA and RNA. Abe et al. [20] developed a biomimetic process to deposit a layer of cryptocrystalline apatitic calcium phosphate on ceramics, metals and polymers immersed, at 36.5–37 °C, in what they called the simulated body fluid (SBF). Mann [21] summarized the biomimetic approaches in inorganic materials chemistry until 1993. However, the examples provided in Mann's article [21] did not show any material synthesis procedures pursued in aqueous media with compositions similar to that of the ECF given in Table 1.

Extracellular fluid is supersaturated with respect to the formation of carbonated (6 wt.% of bones), ion-substituted, non-stoichiometric biological apatite, and the presence and action of biochemical inhibitors of nucleation/crystallization prevents our bodies from being mineralized all over [22]. Blood is circulated in the entire human body, by flowing through the total length of approximately 100,000 km (one hundred thousand kilometers) of veins and capillaries, just to make its inorganic and organic constituents instantly available to the hard and soft tissues, which need to undergo continuous renovation and renewal. This is an outstanding example, given by the human metabolism, to explain the meaning of bioavailability. Calcium phosphate syntheses performed in non-replenished and constant volume batches of distilled/deionized water, which are prone to suffer from the depletion of the initial ions upon the start of precipitation, obviously contradict the scale of bioavailability (of ECF) exemplified by the human metabolism.

Sarikaya and Aksay [23,24] divided biomimetic technology into two categories, i.e. biomimicking (imitating the unique physicochemical and structural design of biomaterials by using the currently available techniques) and bioduplication (mastering the molecular synthesis and processing mechanisms of biological materials to produce new and superior biomaterials), and thus helped to set the roadmap for future research initiatives at the junction of materials science and biology, albeit without mentioning the role of Na^+, Mg^{2+}, Sr^{2+}, SO_4^{2-} and Cl^- present in sea water on mineralization when citing examples on biomineralization of sea urchins and seashells.

Going through the abundant literature related to the synthesis of calcium phosphates in distilled or deionized water has been kept outside the scope of the current review. However, readers may consult the review articles, just to name a few, of Dorozhkin [25,26], Boanini et al. [27], Bleek and Taubert [28], Omelon and Grynpas [29], Wang and Nancollas [30], Coelfen [31], Alves et al. [32], Boskey [33], Addadi and Weiner [34], Habibovic and Barralet [35], Bose and Tarafder [36], Sadat-Shojai et al. [37], and Gomez-Morales et al. [38] for that purpose.

The early, yet quite influential, work of the pioneering calcium phosphate (CaP) researchers, such as Hayek et al. [39,40], Posner et al. [41,42] and Jarcho et al. [43], used distilled water as the synthesis medium and added to it one of the soluble salts of calcium (such as anhydrous $CaCl_2$ (extremely hygroscopic), $CaCl_2{\cdot}2H_2O$, $CaCl_2{\cdot}6H_2O$, $Ca(NO_3)_2{\cdot}4H_2O$ or $Ca(CH_3COO)_2{\cdot}H_2O$) and a water-soluble salt of phosphate (selected from one or more of $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, Na_2HPO_4, $NaH_2PO_4{\cdot}H_2O$, K_2HPO_4 or KH_2PO_4) to induce the precipitation of apatitic CaPs. Such early work, with the notable exception of that of LeGeros et al. [44], did not introduce HCO_3^- ions into the synthesis solutions despite the well-known carbonated nature of bone apatite (5.8 wt.% carbonate). Bone marrow and bones (and most human tissues except hairs and nails) are soaked in blood, not water. Similarly, the external surfaces (whether they are enamel or dentine) of teeth are continuously soaked in saliva but not water.

Early works [39–43] did not mention the possibility of contaminating the Ap-CaP precipitate surfaces with ammonium ions during syntheses, especially when using ammonium phosphate salts, which was shown to be possible much later by the work of Ivanova et al. [45]. Habelitz et al. [46] showed that it would be possible to incorporate nitrogen (even at the wt.% levels) into the lattice of calcium-deficient Ap-CaP at a temperature as low as 800 °C when the heat treatment is performed in an ammonia atmosphere. The earlier articles of the present author on the biomimetic synthesis of Ap-CaP in solutions similar to the ECF also falls in this category since the solutions contained ammonium and nitrate ions which are not present in the ECF [47–50]. The lattice of Ap-CaP is quite accommodating for a significant number of diverse ions (including heavy metals such as lead) present in aqueous solutions in which it was synthesized, as shown in the work of Bigi et al. [27,51,52] and Verbeeck et al. [53].

The majority of published articles related to the synthesis of Ap-CaP [25–38] did not use the main component of blood plasma, i.e. NaCl, in the synthesis solutions, despite Ringer's previous work to that effect [54–56]. On the other hand, when geologists, geochemists and micro- or marine biologists studied the in vitro biomineralization of $CaCO_3$ (calcite, aragonite or vaterite) or apatite, they usually took note of the "salinity" of their mineralization media