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Review article

Tackling bioactive glass excessive *in vitro* bioreactivity: Preconditioning approaches for cell culture tests

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ABSTRACT

Bioactive glasses (BGs) are being increasingly considered for biomedical applications in bone and soft tissue replacement approaches thanks to their ability to form strong bonding with tissues. However, due to their high reactivity once in contact with water-based solutions BGs rapidly exchange ions with the surrounding environment leading in most cases to an undesired increase of the pH under static *in vitro* conditions (due to alkaline ion "burst release"), making difficult or even impossible to perform cell culture studies. Several pre-conditioning treatments have been therefore proposed in laboratories worldwide to limit this problem. This paper presents an overview of the different strategies that have been put forward to pre-treat BG samples to tackle the pH raise issue in order to enable cell biology studies. The paper also discusses the relevant criteria that determine the selection of the optimal pre-treatment depending on the BG composition and morphology (e.g. particles, scaffolds).

Statement of Significance

Bioactive glasses (BGs), since their discovery in 1971 by L.L Hench, have been widely used for bone replacement and repair, and, more recently, they are becoming highly attractive for bone and soft tissue engineering applications. BGs have in fact the ability to form a strong bond with both hard and soft tissues once in contact with biological fluid. The enhanced interaction of BGs with the biological environment is based on their significant surface bioreactivity. This surface effect of BGs is, on the other hand, problematic for cell biology studies by standard (static) cell culture methods: an excessive bioreactivity leads in most cases to a rapid and dramatic increase of the pH of the surrounding medium, which results in cell death and makes cell culture tests on BG samples impossible. The BG research community has been aware of this for many years and numerous pre-treatments have been proposed by different groups worldwide to limit this problem. For the first time, we have reviewed in this paper the variety of surface preconditioning treatments that have been put forward over the years, we provide a summary of such pre-treatments used in laboratory practice, discussing and offering criteria that can be used for the determination of the optimal pre-treatment depending on BG composition and morphology of the sample tested (bulk, particulate, scaffolds). The information and discussion provided in this review should support best research practice when testing bioactive glasses in cell culture.

Contents

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1. Introduction

In the last years the demand for new materials for bone replacement applications has gained continuous importance due to the increase of the average age of the population and the increasing number of surgical procedures [1]. In particular, bone defects above a critical size cannot be repaired by the self-healing of bone tissue and require an osteoconductive and osteoinductive device (scaffold) able to support the regeneration of the new tissue [2]. Although autografts are still considered the 'gold standard', they have many drawbacks such as limited availability and morbidity of the donor site [3]. Xenografts and allografts could be considered a valid alternative, however they have potential drawbacks such as relatively low rates of integration, risk of contamination, immune rejection or viral transmission from the donor [4]. For these reasons, engineered biomaterials are considered highly promising candidates for bone tissue regeneration [2,5–7].

Since first reported in 1971 [8], bioactive glasses (BGs) have been extensively used in bone replacement and repair and, more recently, tissue engineering applications due to their ability to bond *in vivo* to tissues through the development of a biologically equivalent hydroxyl-carbonate-apatite layer, similar to the mineral phase of bone [5,6,9].

The first reported bioactive glass, known as 45S5 Bioglass®, with composition (wt. %): $45SiO_2$-24.5CaO-24.5Na_2O-6P_2O_5, [8], has been used as bulk material for the production of medical devices for dental and orthopaedic applications, as particulate in bone-filler defects, as coating on metallic implants and for fabricating tissue engineering scaffolds [5,6,9–12]. The tissue bonding ability of BGs is based on the high surface reactivity of these materials in contact with aqueous environments. Moreover, a special advantage of BGs is the possibility to tailor their chemical composition by the incorporation of biologically active ions that elicit specific cellular functions [13].

Calcium and phosphorous are the main components of the bone mineral phase and the release of such ions from BGs is relevant in the context of bone tissue engineering applications [9,11]. Moreover silicon, as dissolution product of BGs, is well known to enhance the formation and calcification of the extracellular matrix (ECM) and soluble silica has been shown to contribute to osteoblast activity [10]. Such bioreactivity of BGs has been also considered to be relevant for applications in contact with soft tissues [14].

New glass compositions and/or BGs doped with bioactive ions are being increasingly investigated with the aim to provide the most suitable glass composition for applications in different settings [6,11,14,15]. The biological properties of these new glass compositions have to be evaluated to assess their usability in the respective fields of application. Hence, *in vitro* cell culture studies are always used to analyse the interaction of BGs with cells and to estimate their biological ability, for example regarding the stimulation of osteogenic differentiation or the upregulation of angiogenic growth factors [10,14,16,17].

However, one important issue to consider when BGs get in contact with biological fluids is the development of possible pH-dependent cytotoxicity due to significant changes in localized pH due to an undesired high rate of ion exchange reactions that occur upon interaction of the glass surface with cell culture medium, leading to a burst release. While this effect is not usually observed *in vivo* [6,12], it becomes highly relevant when testing BGs *in vitro*. For this reason, *in vitro* studies to evaluate the behaviour of cells in contact with bioactive glasses must adopt some form of preconditioning of BG samples to limit such non-realistic pH changes. As an example, Fig. 1 shows the pH variation in simulated body fluid (SBF) containing glass powders (45S5 and 58S) with and without pre-conditioning treatment as a function of time [18]. It is shown that pre-conditioning clearly limits the pH excursion without affecting the hydroxyl-carbonate-apatite formation on the powder surfaces, as reported in ref. [18]. Over the years, laboratories around the world have developed a variety of methods and conditioning protocols to pre-treat BGs prior to cell biology studies.

This review summarizes and discusses the different preconditioning methodologies put forward for *in vitro* cell culture characterization of BGs and offers suggestions to select the most efficient, cost effective and fastest method available. Here, we will consider silicate BGs, both sol-gel and met-derived, also discussing different BG morphologies, such as granules, pellets and porous scaffolds.

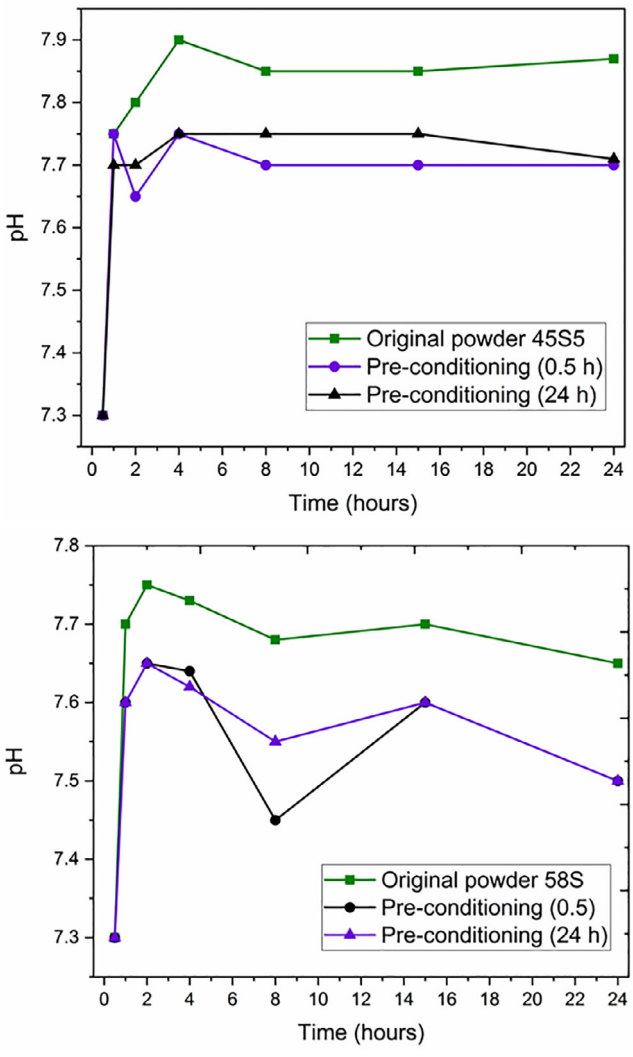

Fig. 1. pH variation of SBF containing bioactive glasses (45S5 and 58S composition) with and without pre-conditioning (adapted from Pryce et al. [18]).

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