Bone and Tissue Regeneration Insights

SHORT COMMENTARY

A Commentary on "Evaluation of the *in vitro* Bioactivity of Bioceramics"

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Abstract: The interesting article reported in Bone and Tissue Regeneration Insights by Wu and Xiao entitled "Evaluation of the *in vitro* bioactivity of bioceramics" contrasts two methods (Simulated body fluid (SBF) and cell culture experiments) which have been commonly used to evaluate the in vitro bioactivity of bioceramics. Limitations in estimating the bioactivity of bioceramics using both methods have been reviewed. Therefore, Wu and Xiao suggest the combination of these two methods to evaluate the bioactivity of bioceramics can improve the screening efficiency for the selection of bioactive ceramics for bone regeneration.

Keywords: *in vitro* test, bioactivity, SBF, cell experiments, bioceramics, apatite formation, osteoconduction

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Introduction

The concept of bioactivity was defined as: "A bioactive material is one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material".[1] It is intermediate between resorbable and bioinert.

Bioceramics, such as bioactive glass, glass-ceramics and calcium phosphate have been widely studied for orthopaedic and dental applications due to their good osteoconductive potential. *In vitro* studies are widely used for the study of bioactive implant materials because such tests allow prediction of the approximate behaviour of such materials *in vivo*.[2] *In vitro* tests are intended for use in screening bone bioactive materials before animal testing. The number of animals used and the duration of animal experiments can be significantly reduced by using these methods, which can assist in the efficient development of new types of bioactive materials.

SBF Method

In 1991, Kokubo[3] proposed that the essential requirement for an artificial material to bond to living bone is the formation of bonelike apatite on its surface when implanted in the living body, and that this *in vivo* apatite formation can be reproduced in simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma.

Thus the ability to form apatite precipitation layers in simulated body fluid (SBF) or in an animal model has been regarded as the evidence of bioactivity for bioceramics and for other types of orthopaedic materials.[4–6] It has been generally accepted that the *in vivo* bone bioactivity of bioceramics can be predicted from the apatite formation on their surfaces in SBF. Kokubo et al[7] also claimed that the SBF method is useful for predicting the *in vivo* bone bioactivity of the material, not only qualitatively but also quantitatively.

However, there are a few materials that directly bond to living bone without the formation of detectable apatite on their surfaces. Both β-TCP and natural calcite exhibit a poor ability to induce calcium phosphate formation on their surfaces in SBF[8,9] or *in vivo*,[9–12] but despite this, they bond to living bone.[10–12] These results might be related to the high resorbability of these materials. Furthermore, the validity of this method for evaluating bioactivity has not been assessed systematically. A range of testing conditions (e.g. specimen shape and dimension, surface roughness, ratio of specimen to solution, buffering agent, immersion periods, static/dynamic test) and SBF solutions have been used by different research groups.[3–7,9,13–26]

Over some decades other research groups have focused their efforts on producing solutions with ion concentrations and pHs as close as possible to human plasma.[27–33] Currently, significant numbers of *in vitro* tests are carried in various forms of SBF solution. The majority utilise a standard pH of 7.25~7.40. However, the ability to induce apatite-like calcium phosphate formation in the various SBF solutions is not directly compared in literature. Additionally, SBF with higher ion concentrations than normal SBF such as 1.5SBF (1.5 time higher ions concentration)[26] or 10SBF (10 times higher)[34] have been used. There is, however, no direct correlation between apatite formation behaviour on a material in normal SBF with that seen in stronger SBFs (1.5 or 10 SBF) or with *in vivo* bone bioactivity.

Recently, a number of tests have been completed in water.[35–39] According to the literature, the results from the tests carried out in water demonstrated dissolution but no precipitation.[36–39] However, precipitates were observed on glass-reinforce hydroxyapatite after three days of immersion in deionised water by Queiroz et al.[35] Similar observations were found in the authors' research work. Figure 1 displays calcium phosphate based samples after immersion in distilled water at pH 7.2 for up to 26 weeks. Precipitates with significantly different morphologies but the same chemical composition were found for the samples after different immersion periods. These results show apatite formation behaviours can be observed in both SBF and distilled water.

In 2009, Bohner and Lemaitre[40] published a review paper entitled "Can bioactivity be tested *in vitro* with SBF solution?" which questioned whether there was currently enough scientific evidence to support the assumptions around the use of the SBF method. The paper concluded that although the use of SBF was valid the variability in the way the tests were carried out left room for improvement. The findings collated by Bohner and Lemaitre[40] indicated that for the most significant mineral bone substitutes used *in vivo* (bioglass, β-TCP, CSH, HA, DCPD), bioactivity testing with SBF may lead not only to false positive but also to false negative results. The authors[40] reported that serum and SBF are supersaturated towards apatite crystals and as such, the system is

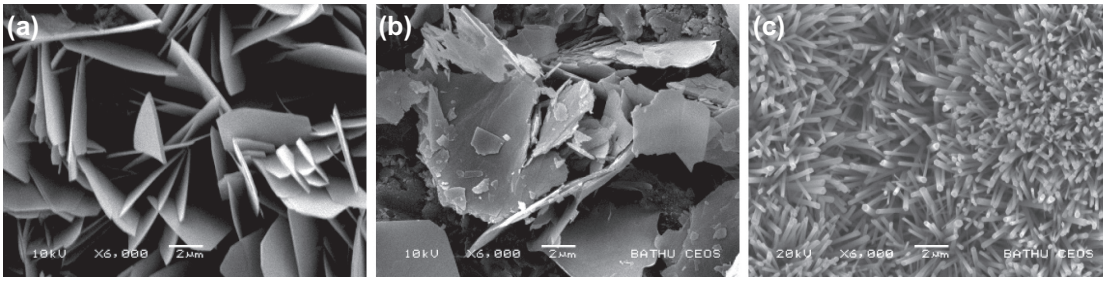

Figure 1. SEM micrographs of surface microstructures of calcium phosphate based bioceramics after immersion in distilled water at pH 7.2 for **a**) 4 **b**) 7 and **c**) 26 weeks.

metastable and will thermodynamically stabilise by the formation of apatite crystals. Consequently, the validity of use of the SBF method to predict the *in vivo* bone bonding ability of a material may be open to question. Bohner and Lemaitre[40] proposed the use of an alternative simplified preparation of SBF solution and that the test should be performed at $p(CO_2) = 0.05$ atm (5%) to replicate the equilibrium state found in human serum.

From reviewing the literature it can be seen that various solutions and SBFs with slightly different compositions have been used by different research groups. The testing conditions vary between groups making direct comparison of the results difficult. In order to be able to compare the results directly, an identical solution and standard testing method need to be established and adopted widely.

Cell Culture Studies

Cell culture experiments are commonly used to evaluate the *in vitro* bioactivity of bioceramics. However, these methods also have a number of limitations which have been well reviewed by Wu and Xiao.[41] As with the SBF studies, the experiments have not been standardised with respect to criteria such as cell type and number, media used, surface roughness of samples, all of which will affect the results recorded.

In conclusion, the two methods reviewed by the authors, SBF and cell culture experiments, have their own drawbacks with respect to the evaluation of the *in vitro* bioactivity of bioceramics. For that reason, Wu and Xiao recommend the combination of SBF and cell experiment methods to evaluate the *in vitro* bioactivity. This is on the basis that, if a novel bioceramic not only has the ability to induce apatite deposition in SBF but also stimulate a cell response, such a bioceramic would most likely also to have excellent *in vivo* bioactivity.

Commentary

The suggestion made by Wu and Xiao might be a useful approach to establish which ceramics demonstrate good bioactivity. However, this selection process may perhaps overlook a potential novel bioceramic which has excellent *in vivo* bone formation ability but does not show a positive response in both SBF and cell experiment methods.

As mentioned before, various testing conditions are used for *in vitro* bioactivity testing. The results from each research group are not directly comparable particularly when predicting the *in vivo* bone bioactivity of the material quantitatively. To this end, standard test methods need to be established both for the SBF and cell culture experiments to allow direct comparison and evaluation of the results.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors report no conflicts of interest.

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