International Standard

ISO 23317

Implants for surgery — Materials — Simulated body fluid (SBF) preparation procedure and test method to detect apatite formation in SBF for initial screening of bone-contacting implant materials

Implants chirurgicaux — Matériaux — Mode opératoire de préparation de fluide corporel simulé (FCS) et méthode d'essai pour détecter la formation d'apatite dans le FCS pour l'étude préliminaire de matériaux d'implant en contact avec l'os

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 150, *Implants or surgery*, Subcommittee SC 1, *Materials*.

This fourth edition cancels and replaces the third edition (ISO 23317:2014), which has been editorially revised. The main changes are:

— the title, Introduction and scope have been revised to clarify the significance and limitations of the SBF test;

— the terms and definitions clause has been rearranged and revised for better understanding;

— the list of apparatus and materials has been enriched and detailed;

— the test specimen preparation has been revised, and test specimen characterization has been added;

— the preparation of SBF has been revised and described in more detail;

— a description of test specimens with lower density than SBF has been added;

— the arrangement of the test specimen in the SBF test has been revised and explained depending on the specimen's shape and density;

— the necessity of visual inspection of SBF has been added;

— the soaking period of seven days in the SBF test has been specified;

— the criteria for judging the specimen's apatite-forming ability in the SBF test have been clarified;

— the test report has been detailed according to the revised SBF test procedure;

— the bibliography has been revised and each bibliographical entry has been cited at the relevant point in this document.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The mechanism of action of bone-contacting implant materials is based upon a complex series of reactions in the body that can be influenced by the surface properties of the implant material in contact with bone. In some cases on synthetic bone-contacting implant materials such as Bioglass, Cerabone® A-W, Ceravital®-type glass-ceramic and sintered hydroxyapatite[1)] ($Ca_{10}(PO_4)_6(OH)_2$), a layer of an avascular, non-cellular apatite-like mineral phase is found on the implant surface, at the bone-implant interface, and is said to promote the bone-bonding behaviour. It has been shown that in vivo apatite formation can be initially modelled in vitro using a number of different aqueous solutions, including an acellular simulated body fluid (SBF) with inorganic ion concentrations nearly equal to those found in human blood plasma[1]-[3].

The apatite formed in an SBF test can indicate if an implant material's physicochemical surface features warrant further evaluation and testing, including cell culture studies and animal studies, to demonstrate safety and efficacy of the implant material[4],[5].

SBF described in this document is highly supersaturated with respect to apatite and several other calcium phosphates and is similar in pH and inorganic ion concentrations to human blood plasma. SBF can retain its metastable state without inducing calcium phosphate precipitation for four weeks under certain, well-controlled conditions described in this document. SBF has been shown to produce a crystalline calcium phosphate (apatite-like) layer that is chemically and crystallographically similar to bone mineral. Thus, SBF can be used as a test solution for initial screening of the formation of calcium phosphate and apatite-like mineral at the surface of a synthetic bone-contacting implant material.

Since SBF can be prepared easily from ultrapure water and ordinary chemical reagents (inorganic salts and a buffer), and the proposed SBF test is a simple and low-cost method available in almost every laboratory, SBF has been used worldwide over the past few decades to evaluate inorganic chemical reactions at the implant surface exposed to the solution. These worldwide tests using SBF have been used to understand biomineralization processes in humans and to be used as a screening tool to predict the potential for in vivo apatite formation on an implant surface. However, SBF is an acellular, biomolecule-free pseudo-physiological solution for mimicking in vivo inorganic chemical reactions only and is used under artificially controlled static conditions. Hence, the SBF test, like other in vitro tests, cannot reproduce in vivo biologically based reactions completely. Some of these limitations are given in the NOTES 1, 2, 3 and 4.

The apatite layer formed in this SBF test can, generally, be detected by conventional surface analytical techniques such as X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX) and Fourier transform infrared spectroscopy (FT-IR). The apatite formed in this SBF test has some similarities to bone mineral (apatite), it is a Ca-deficient type low crystalline apatite which contains the ionic species Mg^{2+}, Na^+, Cl^-, CO_3^{2-}, etc.

NOTE 1 Conditions of the SBF test are different from in vivo conditions in several factors, e.g. lack of biological substances (cells, proteins, etc.), that play a significant role in the ultimate formation of the bone-implant interface, lack of body fluid circulation, lower carbonate and higher chloride concentrations and the presence of tris-hydroxymethyl aminomethane (TRIS) buffer. Note that all these factors affect apatite formation in an SBF test,[6],[7] and can account for the discrepancy between the SBF test results and in vivo results.

NOTE 2 Biological responses (biomolecular events, cellular responses, immunological responses, toxicity, etc.) cannot be evaluated by the SBF test.

NOTE 3 The glass compositions used as reference glasses in this document (in the Na_2O–CaO–SiO_2 glass forming system) have shown a positive correlation between bone-forming ability in a bone defect of a rabbit and apatite-forming ability in this SBF test[8].

1) Bioglass, Cerabone® A-W, Ceravital®-type glass-ceramic and sintered hydroxyapatite are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

NOTE 4 The relationship between the in vitro formation of an apatite-like mineral layer as proposed in this document and the ultimate in vivo response of the implant material is not direct and is subject to many variables. Bioglass (45S5 and other glasses in this series),[1]-[3],[8] CaO-SiO_2 glasses,[9] Cerabone® A-W,[10] Ceravital®-type glass-ceramic,[10] sintered hydroxyapatite[10] and alkali and heat treated titanium metal,[11] all have shown to bond to bone most likely through an apatite layer developed at the bone–implant interface in vivo and all form an apatite-like mineral layer on their surfaces in an SBF test.[12]-[20] However, there are materials with relatively high solubility such as beta-tricalcium phosphate ($Ca_3(PO_4)_2$)[21] and calcium carbonate[22] that can bond to bone without forming an apatite layer on their surfaces, either in vitro (in an SBF test) or in vivo. Apatite formation in this test is a result of chemically driven calcium phosphate precipitation, crystallization and growth. Some material formulations resorb too quickly to form a direct bond to living bone, such as calcium sulfate hemihydrate, calcium sulfate dihydrate and dicalcium phosphate dihydrate; but they can form an apatite-like layer on their surfaces in an SBF test.[6] In addition, even toxic materials and materials known to cause inflammatory responses upon implantation in bone can form an apatite-like layer when soaked in SBF. Therefore, this document can only be used for initial screening of implant materials to evaluate their potential use in bone implantation sites.

Implants for surgery — Materials — Simulated body fluid (SBF) preparation procedure and test method to detect apatite formation in SBF for initial screening of bone-contacting implant materials

1 Scope

This document specifies a procedure for preparing the simulated body fluid (SBF) and a test method for use as an initial screening tool in the evaluation of apatite formation on the surfaces of bone-contacting implant materials.

NOTE 1 The results of this SBF test (see Clause 7) alone do not establish bone-bonding ability. The test can be used along with other in vitro and in vivo confirmatory tests to establish an implant material's ability to bond with bone tissue in vivo.

This document is limited to an assessment of the in vitro apatite-forming ability of bulky solid materials used for bone-contacting implants and is not intended to be used to evaluate this ability of porous materials, particulate materials or solute molecules or ions.

NOTE 2 Porous materials are excluded from test specimens because they require a large volume of SBF due to high surface area, and often have difficulty in penetration of SBF into their porous bodies. Furthermore, analysis of the inner surfaces of porous materials is difficult by the method described in this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3611, *Geometrical product specifications (GPS) — Dimensional measuring equipment — Design and metrological characteristics of micrometers for external measurements*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 13385-1, *Geometrical product specifications (GPS) — Dimensional measuring equipment — Part 1: Design and metrological characteristics of callipers*

ISO 13385-2, *Geometrical product specifications (GPS) — Dimensional measuring equipment — Part 2: Design and metrological characteristics of calliper depth gauges*

ISO 14630, *Non-active surgical implants — General requirements*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 14630 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/