INTERNATIONAL STANDARD

ISO 23317

Third edition
2014-06-15

Implants for surgery — In vitro evaluation for apatite-forming ability of implant materials

Implants chirurgicaux — Évaluation in vitro de la capacité de formation d'apatite des matériaux d'implants

Reference number
ISO 23317:2014(E)

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Published in Switzerland

Contents

Page

Foreword

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The committee responsible for this document is ISO/TC 150, *Implants for surgery*, Subcommittee SC 1, *Materials*.

This third edition cancels and replaces the second edition (ISO 23317:2012), which has been editorially revised.

Introduction

It has been revealed that materials of various kinds bond to living bone through a layer of apatite. It has been shown that this apatite layer can be reproduced on their surfaces in an acellular and protein-free simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma, and that apatite thus formed is similar to the bone mineral in its composition and structure.

This evaluation of apatite-forming ability on implant material in SBF is useful for evaluating its *in vivo* bone-bonding ability preliminary to animal experiments. When a bioactive material is implanted in a living body, a thin layer rich in Ca and P forms on its surface. The material then connects to the living tissue through this apatite layer without a distinct boundary. It has been shown that this apatite layer can be reproduced on the surfaces of materials in SBF as well, and that apatite thus formed is similar to bone mineral in its composition and structure. As bioactivity increases, apatite forms on the material surface in a shorter time in proportion to this increase. The formation of apatite layers can be detected by thin film X-ray diffraction spectrometry and/or scanning electron microscopy.

The apatite formed in the SBF is, however, similar to bone apatite in the following points.

— Ca-deficient type apatite.

— Lower Ca/P atomic ratio than stoichiometric apatite.

— Containing some impurities such as Mg^{2+}, Na^{+}, Cl^{-}, HCO_3^{-}.

— Low crystallinity.

NOTE 1 The material which forms apatite on its surface *in vivo* can bond to living bone, since this apatite is biologically active. Their *in vivo* apatite deposition can be reproduced on their surfaces even *in vitro* in SBF. For example, *in vivo* calcification on surfaces of Bioglass®[1], $CaO\text{-}SiO_2$ glasses, $Na_2O\text{-}CaO\text{-}SiO_2$ glasses, Cerabone®[2] A-W, Ceravital®[3] -type glass-ceramic, sintered hydroxyapatite and alkali-heat-treated titanium metal, are correlated with *in vitro* calcification in SBF. However, this does not exclude the possibility that materials, which do not form apatite on their surfaces *in vivo*, bond to living bone. For example, it is reported that such resorbable materials as beta-tricalcium phosphate ($Ca_3(PO_4)_2$) and calcium carbonate bond to living bone without forming an apatite layer on their surfaces.

NOTE 2 It has been reported that glasses with different compositions in the system $Na_2O\text{-}CaO\text{-}SiO_2$ show a correlation between bone-forming ability of materials implanted into a bone defect of a rabbit and apatite-forming ability on its surface in SBF.

1) Trade names of products are an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

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Implants for surgery — In vitro evaluation for apatite-forming ability of implant materials

1 Scope

This International Standard specifies a method for detecting apatite formed on a surface of a material in simulated body fluid (SBF). It is applicable to implant surfaces intended to come into direct bone contact.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

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.

3.1
apatite
group of calcium-phosphates including bone mineral and the main inorganic constituent of bones and teeth similar to hydroxyapatite, which has the composition $Ca_{10}(PO_4)_6(OH)_2$

Note 1 to entry: Bone mineral also contains ions such as CO_3^{2-}, F^-, Na^+ and Mg^{2+}.

3.2
apatite-forming ability
capability to develop apatite on the surface

3.3
bioactivity
property that elicits a specific biological response at the interface of the material, which results in the formation of a bond between tissue and material

3.4
induction period
time to detect apatite formation on a surface of a specimen after soaking the specimen in simulated body fluid

3.5
simulated body fluid
SBF
inorganic solution having a similar composition to human blood plasma without organic components

3.6
standard glass for evaluating apatite-forming ability
class of standard glasses with certain chemical compositions as shown in Annex B showing given apatite-forming abilities in SBF and when implanted in an animal body

3.7
thin film X-ray diffraction spectrometry
TF-XRD
method for detecting minerals in a thin layer at the surface of a material from a diffraction pattern obtained by X-ray with small glancing angle against the surface of the sample

4 Apparatus

4.1 Electric balance, capable of measuring a mass with an accuracy of ± 1 mg.

4.2 Water bath equipped with magnetic stirrer, to maintain temperature of the solution within the range of (36,5 ± 2) °C and an accuracy of ± 0,2 °C.

4.3 pH meter, capable of measuring the pH of a solution with an accuracy of ± 0,01.

4.4 Thermometer, capable of measuring the temperature of a solution with an accuracy of ± 0,1°C.

4.5 Thin film X-ray diffraction spectrometer (TF-XRD), capable of detecting apatite formed in a thin layer at the surface of a material.

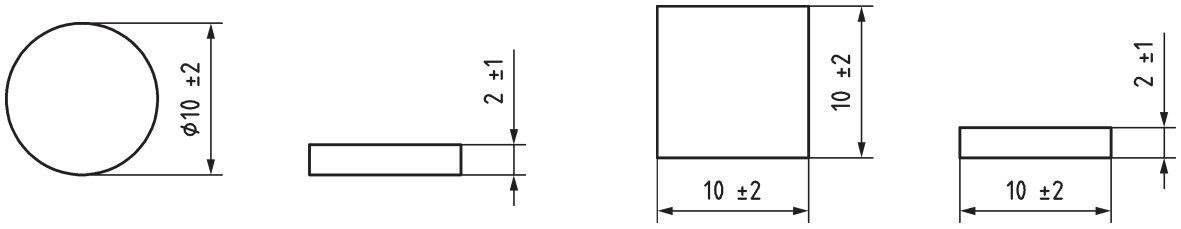
4.6 Scanning electron microscope (SEM), capable of observing apatite grains and/or layers formed on a plain surface of a material with a magnification up to × 10 000.

5 Test specimen

5.1 Specimen configuration and size

This International Standard allows specimens of any configuration and size derived from implant parts and devices to be used. However, a disc or rectangular plate specimen is highly recommended, because bioactivity of a material is evaluated by confirmation of apatite formed on the surface of the material using TF-XRD and/or SEM. Recommended specimen dimensions are shown in Figure 1.

Dimensions in millimetres



a) Disc specimen **b) Rectangular specimen**

Figure 1 — Recommended specimen dimensions

5.2 Specimen preparation

5.2.1 General

This International Standard allows several options for specimen preparation. The specimens should be machined, if necessary, to alter the configurations of original implants.

5.2.2 Basic machining procedure

In the case of a rectangular thin plate specimen as shown in Figure 1 b, the following procedure shall be used. Specimens shall be ground using a diamond wheel of grit size between 120 and 400. Conditions such as depth of cut per pass, wheel speed and others depend on the ground material. Water soluble materials, such as bioactive standard glasses, shall be machined under non-aqueous conditions.

Where a customary machining procedure has been developed that is completely satisfactory for apatite-forming ability testing, this customary procedure can be used.

6 Simulated body fluid

6.1 General

Simulated body fluid (SBF) as defined in Table 1 shall be used.

Table 1 — Ion concentrations of SBF and human blood plasma

Ion	Concentration (10^{-3} mol) in	
	SBF (pH 7,40)	**Blood plasma** (pH 7,2 to 7,4)
Na^+	142,0	142,0
K^+	5,0	5,0
Mg^{2+}	1,5	1,5
Ca^{2+}	2,5	2,5
Cl^-	147,8	103,0
HCO_3^-	4,2	27,0
HPO_4^{2-}	1,0	1,0
SO_4^{2-}	0,5	0,5

NOTE 1 For SBF as defined in Table 1, a correlation was observed between *in vivo* bone ingrowth and *in vitro* apatite-forming ability.

NOTE 2 Other kinds of SBFs have been proposed in the literature, some of which have shown a correlation between *in vivo* bone ingrowth and *in vitro* apatite-forming ability.

6.2 Reagents for SBF

For the preparation of SBF only reagents of the following recognized analytical grade chemicals and only water in accordance with ISO 3696:1987, grade 2, shall be used.

6.2.1 Sodium chloride (NaCl)

6.2.2 Sodium hydrogen carbonate ($NaHCO_3$)

6.2.3 Potassium chloride (KCl)

6.2.4 Di-potassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$)

6.2.5 Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)

6.2.6 Hydrochloric acid solution, c(HCl) = 1 mol/l.

6.2.7 Calcium chloride ($CaCl_2$) or calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)

6.2.8 Sodium sulfate (Na_2SO_4)

6.2.9 Tris-hydroxymethyl aminomethane (TRIS): $((HOCH2)_3CNH_2)$

6.3 Preparation of SBF

6.3.1 General

Since SBF is supersaturated with respect to apatite, an inappropriate preparation method can lead to the homogeneous precipitation of apatite in the solution.

During its preparation the solution shall remain colourless, transparent and without deposit on the surface of the bottle. If any precipitation occurs, stop preparing SBF, abandon the solution and restart by washing the apparatus.

In Table 2, the reagents for the preparation of 1 l of SBF are given in the required order of dissolution.

Table 2 — Ion concentrations of SBF and human blood plasma

Order	Reagent	Amount [a]	Container	Purity [b]	Formula weight
1	6.2.1	8,035 g	weighing paper	99,5 %	58,443 0
2	6.2.2	0,355 g	weighing paper	99,5 %	84,006 8
3	6.2.3	0,225 g	weighing bottle	99,5 %	74,551 5
4	6.2.4	0,231 g	weighing bottle	99,0 %	228,222 0
5	6.2.5	0,311 g	weighing bottle	98,0 %	203,303 4
6	6.2.6	39 ml	graduated cylinder	—	—
7	6.2.7 [c]	0,292 g	weighing bottle	95,0 %	110,984 8
8	6.2.8	0,072 g	weighing bottle	99,0 %	142,042 8
9	6.2.9	6,118 g	weighing paper	99,0 %	121,135 6
10	6.2.6	0 ml to 5 ml	syringe dropper	—	—

[a] The amounts of the reagents are changed depending upon their purities.

[b] The purity given in this table is a typical purity for reagent available in most countries.

[c] If calcium chloride dihydrate ($CaCl_2$) • $2H_2O$ is used, attention shall be given to the different molar weight:

– amount 0,371 g

– purity 99,0 %

– formula weight 147,015 2

6.3.2 Step 1

Put 700 ml of ion-exchanged and distilled water, with a stirring bar, into a 1 litre plastic beaker. Set it in the water bath (4.2) on the magnetic stirrer and cover it with a watch glass or plastic wrap. Heat the water in the beaker to (36,5 ± 1,5)°C while stirring. Annex A shows an example of an apparatus for preparing the SBF.

6.3.3 Step 2

Dissolve the 1st to 8th reagents in the required order given in Table 2 in the distilled water at (36,5 ± 1,5)°C, while considering the following.

a) Glass containers should be avoided. A plastic container, with a smooth surface and without any scratches, is recommended, because apatite nucleation can be induced at the surface of a glass container or the edges of scratches.

b) Dissolve a reagent only after the preceding one (if any) is completely dissolved.

c) Dissolve the $CaCl_2$/$CaCl_2 \cdot 2\,H_2O$ little by little as the reagent has a great effect on the precipitation of apatite.

d) Rinse the graduated cylinder with 1 mol/l HCl before measuring the volume of 1 mol/l HCl.

e) Measure the hygroscopic reagents such as $K_2HPO_4 \cdot 3\,H_2O$, $MgCl_2 \cdot 6\,H_2O$, $CaCl_2$/$CaCl_2 \cdot 2\,H_2O$, KCl, Na_2SO_4 as quickly as possible.

6.3.4 Step 3

Insert the electrode of the pH meter (4.3) into the solution. Just before dissolving the TRIS, the pH of the solution should be 2,0 ± 1,0.

6.3.5 Step 4

Set the temperature of the solution at (36,5 ± 1,5)°C. If the amount of the solution is smaller than 0,9 l, add distilled water up to 0,9 l in total.

6.3.6 Step 5

With the solution temperature between (36,5 ± 1,5)°C, preferably (36,5 ± 0,5)°C, dissolve TRIS into the solution little by little, taking careful note of the pH change. After adding a small amount of TRIS, wait until the reagent is dissolved completely and the pH has become constant. Then add another small amount of TRIS.

It is recommended not to add a large amount of TRIS into the solution all at once, because the radical increase in local pH of the solution could lead to the precipitation of apatite. The following procedure is recommended: If the solution temperature is not within (36,5 ± 0,5)°C, add TRIS to raise the pH to 7,3 ± 0,05, then stop adding and wait for the solution temperature to reach (36,5 ± 0,5)°C. With the solution at (36,5 ± 0,5)°C, add more TRIS to raise the pH to under 7,45. The pH should not increase to over 7,45 at (36,5 ± 0,5)°C, taking account of the pH decrease with increasing solution temperature.

6.3.7 Step 6

Make sure that the temperature of the solution is maintained at (36,5 ± 0,5)°C. When the pH has risen to 7,45 ± 0,01, stop dissolving TRIS, then add HCl solution by syringe to lower the pH to 7,42 ± 0,01, taking care that the pH does not decrease below 7,40. After the pH has fallen to 7,42 ± 0,01, dissolve the remaining TRIS little by little until the pH has risen to ≤ 7,45. If any TRIS remains, add the 1 mol/l -HCl and TRIS alternately into the solution. Repeat this process until the whole amount of TRIS is dissolved keeping the pH within the range of 7,42 to 7,45. After dissolving the whole amount of TRIS, adjust the temperature of the solution to (36,5 ± 0,2)°C. Adjust the pH of the solution by adding HCl solution little by little at a pH of 7,42 ± 0,01 at (36,5° ± 0,2)°C and then finally adjust it to 7,40 exactly at 36,5°C on condition that the rate of solution temperature increase or decrease is less than 0,1°C/min.

6.3.8 Step 7

Remove the electrode of the pH meter from the solution, rinse it with distilled water and add the washings to the solution.