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Tissue-engineered medical products — Bioactive ceramics — Method to measure cell migration in porous materials

Produits médicaux issus de l'ingénierie tissulaire — Céramiques bioactives — Méthode de mesure de la migration cellulaire dans les matériaux poreux

Reference number
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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 documents 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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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

ISO 19090 was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 7, *Tissue-engineered medical products*.

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Introduction

"Bioactive ceramics" are widely used in orthopaedic and dental fields due to their bioactivities and bioaffinities. Porous bioactive ceramics are designed as bone void fillers, and cell migration from tissue into their pores is an expectation for effective repair of bone defects; thus, they are one of the promising candidates for cell scaffolds for bone tissue engineering medical products.

To clarify the clinical safety and usefulness of these bioactive ceramics, physical, chemical and biological properties must be examined. In the methods used, animal tests are the ultimate and essential methods to examine biological properties of bioactive ceramics; however, numbers of both animals and animal tests must be reduced under the concept of 3R (Replacement, Reduction and Refinement)[3].

The first and most important property for porous biomaterials including bioactive ceramics is cell migration capability, because cell proliferation, differentiation, tissue formation and tissue maturation in and surroundings of porous biomaterials do not occur without cell migration.

Currently, two different cell-seeding methods are used for estimating "cell migration" property: One is dropping a cell suspension on the top surface of a porous material. This method tests the penetration ability of the "cell suspension" under gravity and estimates the number of cells that migrate into and are held within the porous material. The other method is shaking a porous material in the cell suspension. This method also tests the penetration ability of the "cell suspension" like the above method but uses shaking to drive the cells into the porous scaffolds. Both methods test the abilities of cell penetration and retention only, and do not test the intrinsic ability of the cell to migrate simulating what happens *in vivo*. Body fluid itself can sufficiently carry cells across a minor gap between the implanted material and the host bone. Accordingly, no cell migration test methods have been reported that mimic cell behaviour *in vivo*.

When porous bioceramics are implanted into bone defect, cells migrate into the pore to form new bone. In this process, migration of osteoblasts mainly plays important roles for osteoconduction. That is to say, no osteoconduction nor bone formation can occur without osteoblast migration.

Therefore, it is imperative to establish a quantifiable method to measure cell migration potential of porous bioactive ceramics in a manner similar to how cells behave *in vivo*, in order to evaluate their potential appropriately as materials for tissue-engineered medical products.

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Tissue-engineered medical products — Bioactive ceramics — Method to measure cell migration in porous materials

1 Scope

The document specifies the test method to be followed for measuring and documenting the cell migration ability of porous bioactive ceramic materials.

This document is not applicable to porous materials that have low or no cell adhesion properties, for instance synthetic polymers and metals. These types of materials will require longer times to allow effective transfer and migration of cells from the cultured substrate to the test specimen. To minimize influences of cell passages, cell kinds, differences in cell culture consumables including culture medium and fetal bovine serum etc., the method uses a porous bioactive ceramics, which is clinically and widely used in each country, as a reference material for calculation of relative migration distance.

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 10993-5, *Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10993-5 and the following apply.

ISO and IEC maintain terminological 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/

3.1
bioceramics
ceramics used that enhance biological functions when implanted in the human body

3.2
bioactive ceramics
bioceramics (3.1) with direct bone bonding property when implanted into bone defect

3.3
biomaterial
material used in or to be used in medical and dental field

3.4
full confluent
cell cultured dish is almost completely (≈95 % to 100 %) covered with a monolayer of cells

3.5
complete medium
cell culture media that is recommended for the chosen cell type by the supplier of the cells with all required supplements cell culture medium that is confirmed by the user that the cells used in the test proliferate well without any mutation

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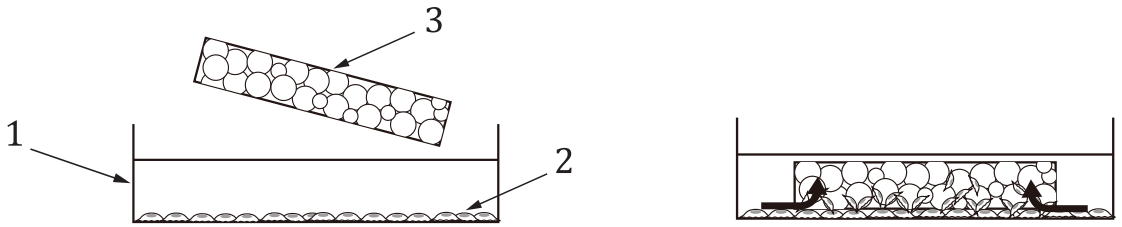
3.6
osteoblast-like cell
established cell lines which widely recognised to have “osteogenic activity”

3.7
cell group
group of cells composed of at least five cells where the distance between each cell is less than the cell width

4 Principle

The cell migration property of porous biomaterials has been estimated by measuring cell numbers that are seeded in the porous body by two methods, however, they measure penetration of cell suspension and cell attachment, not migration by cells themselves.

The present method is very simple and effective to measure cell migration by themselves. Cells confined in a confluent layer of a culture dish are able to further migrate and proliferate from this layer into the porous bioceramic materials that are placed on top of the culture dish as shown in Figure 1. To minimize influences of cell passages, cell types, differences in cell culture consumables including culture medium and serum, etc., the method uses a reference material that porous bioactive ceramics commercialised and used clinically with good clinical results in each country for calculation of relative migration distance.



a) Before: No cells exist in a test material

b) After: Cells move up to a test material through pores

Key

1 culture dish filled with cell culture medium
2 cells adhered on a bottom of culture dish
3 test material

Figure 1 — Schematic drawing of test method

After the initial transfer of cells onto the material interface, they start to migrate into pores of the materials. This migration distance will differ in relation to the materials properties and chemical stability, surface morphologies, and pore structures similar to what is seen *in vivo* and which mimics the initial part of the bone regeneration process.

Giemsa staining is a very stable and easy method to stain cells for this method.

Linear longest migration distance of a valid cell measured from the cross section of the Giemsa stained porous bioactive ceramics is well reflected cell migration *in vivo*.

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