In Vivo Cryotechnique in Biomedical Research and Application for Bioimaging of Living Animal Organs

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Preface

We medical and biological scientists want to know the real in vivo morphology and also the immunolocalizations of all molecular components in functioning cells and tissues of living animal organs; we need the "living animal morphology," not the dead (nor conventionally prepared with fixatives) animal one. Recently, the live imaging of cells and tissues of animals with fluorescence-labeled proteins by gene manipulation has become increasingly popular in biological fields. For those reasons, we should now examine the histological or immunohistochemical tissue sections of living animal organs which must be exactly compatible with their live imaging, usually clarified by recently developed digital imaging techniques.

In our opinion, the already developed immunohistochemical or morphological research performed by in vivo cryotechnique (IVCT) depends exclusively on functioning cells and tissues of living animal organs, so it is now necessary to publish a new, innovative book, reviewing especially the real morphofunctional findings in vivo of cells and tissues of living animal organs. However, until now there has been no such book about living animal morphology on permanent tissue section preparations corresponding to the digital live imaging of fluorescence-labeled soluble or structural molecules.

In the past few decades, we have developed an original IVCT to capture the dynamically changing morphology and immunolocalizations of signal molecules and receptors of cells and tissues in addition to detecting soluble probes of living animal organs, reported in more than 60 biomedical papers in the last 20 years. Therefore, the publication of such an innovative review focusing on our research field is both significant and timely.

We hope that this volume will provide many readers with a good opportunity to learn about IVCT, including cryobiopsy, for their future studies. Finally, we are grateful to all the authors who have contributed to this publication.

Yamanashi, Japan — Shinichi Ohno
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Contents

Part I
Overview

Introduction

1

Shinichi Ohno

Abstract

Morphological studies with light or electron microscopy and scanning probe microscopy have been major approaches to understand physiological and pathological features of living animal organs. Although other rapid progresses of research techniques in molecular or genetic biology have been realized to establish new molecular or biological fields, morphological techniques are still necessary for more precise understanding of living animal organs. For routine morphological analyses, both chemical fixation and alcohol dehydration have been commonly used to keep cells and tissues, but they always bring about many morphological artifacts, including tissue shrinkage and extraction of components. To the contrary, both quick-freezing and high-pressure freezing methods of resected fresh tissues have been also contributed to reduction of such morphological artifacts, but they have to be resected from living animal organs with blood supply. The in vivo cryotechnique (IVCT) has been found to be extremely useful to arrest transient physiological processes of cells and tissues and also to maintain their intra- and extracellular components in situ. The IVCT has already allowed us to perform novel morphological investigations of cells and tissues in living animal organs and will further contribute to new medical and biological fields with "living animal morphology."

Keywords

Microscopy • Conventional fixation • Quick-freezing • In vivo cryotechnique • Soluble components

The morphological study with light or electron microscopy in addition to scanning probe microscopy has been one of the major approaches to understand physiological and pathological features of living animal organs in medical and biological fields. Especially, the conventional electron microscopy, developed in the last half of the twentieth century, greatly facilitated the enormous progress of ultrastructural analyses of cells and tissues, which has been now indispensable in morphological field with many applications. Although other rapid progresses of research techniques in molecular or genetic biology have been realized to establish new molecular or biological fields in the past few decades, various morphological techniques are still necessary for more precise understanding of cells and tissues in living animal organs. In such approach cases, their obtained structures should always reflect some functional aspects of the living animal organs.

For the routine morphological analyses, both chemical fixation and alcohol dehydration have been commonly used as easy preparation procedures to keep cells and tissues, but they always bring about many morphological artifacts, including tissue shrinkage and extraction of components, of dynamically changing organs in vivo (Fig. 1.1a, b) [1–3]. To

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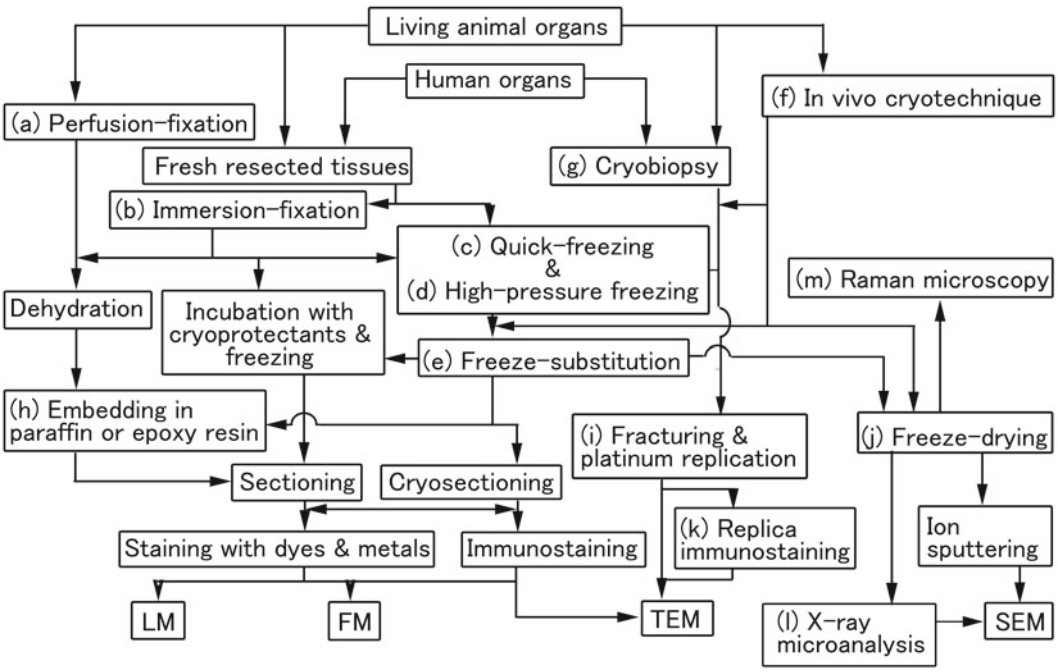

Fig. 1.1 A flowchart of various preparation steps for light microscopy (*LM*), fluorescence microscopy (*FM*), transmission electron microscopy (*TEM*), and scanning electron microscopy (*SEM*). The perfusion- (**a**) or immersion- (**b**) fixation and dehydration steps, the quick-freezing (**c**) and high-pressure freezing (**d**) methods, and the "in vivo cryotechnique" (**f**) are described with connection with their following preparation steps. Note that all preparation steps, following the quick-freezing and high-pressure freezing methods, are also available after the "in vivo cryotechnique" (**f**). To apply the "in vivo cryotechnique" to human organs, a new cryotechnique of biopsy, termed as "cryobiopsy" (**g**), would be necessary, as described in the new cryobiopsy chapter

the contrary, both conventional quick-freezing and high-pressure freezing methods of resected fresh organ tissues, by which they are quickly frozen for physical fixation, can be also contributed to reduction of such morphological artifacts (Fig. 1.1c, d) [4, 5], but they have to be resected from living animal organs with blood supply for the freezing. Therefore, those animal specimens are inevitably exposed to stresses of ischemia and anoxia, exhibiting only dead morphological states of animal organ tissues without normal blood circulation. For the past years, the original "in vivo cryotechnique" was also developed to demonstrate new dynamically changing morphology and immunolocalizations of functional proteins in cells and tissues at light or electron microscopic levels (Fig. 1.1f) and clarify morphofunctional significance of cells and tissues in living animal organs [6–8], as described in the next paragraph.

As everyone knows, the final goal of morphological and immunohistochemical studies in our biological or medical fields is that all findings examined in animal experiments should reflect the physiologically functional background. Therefore, the preservation of all original components in targeted cells and tissues of animals is necessary for describing the functional morphology of living animal organs. It has been generally accepted that morphological findings of various animal organs were easily modified by stopping their blood supply, because of ischemia or anoxia. There had been a need to develop a new preparation technique for freezing the living animal organs in vivo and then obtaining their acceptable morphology and also immunolocalizations of original soluble components in functioning cells and tissues. We have already developed the "in vivo cryotechnique" (IVCT) not only for their morphology, but also for immunohistochemistry of many soluble components in various living animal organs [6–9]. All physiological processes of them were immediately immobilized in the vitreous ice by IVCT, and every component in the cells and tissues was maintained in situ at the time of freezing. Thus, the ischemic or anoxic artificial effects on them could be minimized by the newly developed IVCT. Our specially designed liquid cryogen system with or without a cryoknife has totally solved the morphological and immunohistochemical problems which are inevitable by the conventional preparation methods at a light or electron microscopic level [6, 8]. The IVCT has been found to be extremely useful to arrest transient physiological processes of cells and tissues and also to maintain their intra- and extracellular components in situ, such as rapidly changing signal molecules, membrane channels, and receptors, as described before [8, 9].

As described above, the IVCT has already allowed us to perform novel morphological investigations of cells and tissues in living animal organs and will further contribute to new medical and biological fields with "living animal mor-