Available online at www.sciencedirect.com
SCIENCE DIRECT®
Progress in Biophysics and Molecular Biology 92 (2006) 232–257
Progress in Biophysics & Molecular Biology
www.elsevier.com/locate/pbiomolbio

Review

Macroscopic optical mapping of excitation in cardiac cell networks with ultra-high spatiotemporal resolution

Emilia Entcheva[a,b,*], Harold Bien[a]

[a]*Department of Biomedical Engineering, Stony Brook University, HSC T18-030, Stony Brook, NY 11794-8181, USA*
[b]*Department of Physiology and Biophysics, Stony Brook University, Stony Brook, NY 11794, USA*

Available online 21 November 2005

Abstract

Optical mapping of cardiac excitation using voltage- and calcium-sensitive dyes has allowed a unique view into excitation wave dynamics, and facilitated scientific discovery in the cardiovascular field. At the same time, the structural complexity of the native heart has prompted the design of simplified experimental models of cardiac tissue using cultured cell networks. Such reduced experimental models form a natural bridge between single cells and tissue/organ level experimental systems to validate and advance theoretical concepts of cardiac propagation and arrhythmias. Macroscopic mapping (over $>1\,cm^2$ areas) of transmembrane potentials and intracellular calcium in these cultured cardiomyocyte networks is a relatively new development and lags behind whole heart imaging due to technical challenges. In this paper, we review the state-of-the-art technology in the field, examine specific aspects of such measurements and outline a rational system design approach. Particular attention is given to recent developments of sensitive detectors allowing mapping with ultra-high spatiotemporal resolution (>5 megapixels/s). Their interfacing with computer platforms to match the high data throughput, unique for this new generation of detectors, is discussed here. This critical review is intended to guide basic science researchers in assembling optical mapping systems for optimized macroscopic imaging with high resolution in a cultured cell setting. The tools and analysis are not limited to cardiac preparations, but are applicable for dynamic fluorescence imaging in networks of any excitable media.

Keywords: Optical mapping; Cultured cells; Fluorescent probes; Calcium; Transmembrane potentials

Contents

*Corresponding author. Department of Biomedical Engineering, Stony Brook University, HSC T18-030, Stony Brook, NY 11794-8181, USA. Tel.: +1 631 444 2368; fax: +1 631 444 6646.
E-mail address: emilia.entcheva@sunysb.edu (E. Entcheva).

0079-6107/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pbiomolbio.2005.10.003

1. Introduction

Excitation waves are complex spatiotemporal phenomena encoding essential functional information for healthy and diseased excitable tissue, including the heart. The visualization of these waves in live tissue was facilitated by the introduction of fast fluorescent probes for changes in transmembrane voltage and intracellular calcium concentration, and by the development of appropriate optical techniques to image their response (Salama and Morad, 1976; Grinvald et al., 1977; Ross et al., 1977; Morad and Salama, 1979; Gross et al., 1986; Ehrenberg et al., 1987; Tsien, 1983; Grynkiewicz et al., 1985a). Since then, *optical mapping* (multi-site fluorescence measurements with high temporal and spatial resolution) has made possible the direct experimental testing of theoretical concepts about cardiac arrhythmias, cardioversion and electrical excitation in the heart. Optical mapping in culture-grown monolayers or patterns of myocytes allows the study of cellular processes in their natural context, avoiding some of the deficiencies associated with the two extremes: isolated cells or whole heart measurements. It permits the true dissection of propagation phenomena and direct links to computational models of the same by controlled local or global alterations of structural and functional properties—a feature not readily available in whole heart or tissue preparations. Thus, cultured cardiomyocyte networks form a natural bridge between single cell and whole heart studies in cardiac electrophysiology.

Optical *microscopic mapping in cardiomyocyte cultures* was pioneered by Rohr, Fast and Kleber at the University of Bern, employing patterned cell growth (Rohr et al., 1991) and custom-developed imaging system using a fluorescence microscope, photodiodes and optical fibers (Rohr and Salzberg, 1994; Rohr and Kucera, 1998). In a series of elegant optical mapping studies, this group and their collaborators addressed questions of load mismatch in structurally complex cell network architectures (Fast and Kleber, 1993, 1995a, b; Rohr et al., 1997), cell-level polarization patterns in response to external electrical fields in cardioversion and defibrillation (Fast et al., 1998, 2004; Gillis et al., 1996, 2000; Tung and Kleber, 2000; Fast and Ideker, 2000), micro-reentrant phenomena in slow propagation conditions (Kucera et al., 1998; Rohr and Kucera, 1997), etc.

Understanding cell network behavior at the *macroscopic scale* and the study of phenomena underlying dangerous cardiac arrhythmias required the extension of this imaging approach to accommodate a larger field of view (FOV). Signature reentrant waves, believed to be at the core of cardiac arrhythmias, are macroscopic spatiotemporal phenomena, taking place over a spatial scale that is linked to the wavelength for propagation ($\lambda_w = \theta w$, where θ is the wave's conduction velocity and w signifies the duration of the events of interest—action potentials or calcium transients). For typical values of θ and w, the spatial scale of interest is in the centimeter range, thus requiring a matching FOV in that range. Due to technical difficulties and limitations of optical imaging at low magnification in low light levels, the transition from micro- to macroscale mapping in monolayer cell cultures is not trivial, i.e. is not as simple as changing an objective.

The first attempts at *macroscale mapping of cardiac electromechanics in cultured cells* (voltage or calcium waves) originated in three laboratories. Bub, Shrier and Glass at McGill University (Bub et al., 1998, 2002, 2003) used a charge-coupled device (CCD)-based system to track the dynamics of spontaneous and induced spiral waves as a function of cell density and age in cultured embryonic chick cells. Tung lab at Johns Hopkins University (Entcheva et al., 2000, 2004b; Iravanian et al., 2003) developed a contact fluorescence imaging (CFI) approach combining photodiodes and fiber optics to study anatomical and functional reentry in neonatal rat cultures. Sarvazyan lab at Texas Tech University (Arutunyan et al., 2001, 2002) used a confocal system to assess calcium dynamics in reperfusion injury in cultured cell networks with a geometrically defined