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Review article

Fibroblast–myocyte electrotonic coupling: Does it occur in native cardiac tissue? ☆

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ABSTRACT

Heterocellular electrotonic coupling between cardiac myocytes and non-excitable connective tissue cells has been a long-established and well-researched fact *in vitro*. Whether or not such coupling exists *in vivo* has been a matter of considerable debate. This paper reviews the development of experimental insight and conceptual views on this topic, describes evidence in favour of and against the presence of such coupling in native myocardium, and identifies directions for further study needed to resolve the riddle, perhaps less so in terms of principal presence which has been demonstrated, but undoubtedly in terms of extent, regulation, patho-physiological context, and actual relevance of cardiac myocyte–non-myocyte coupling *in vivo*. This article is part of a Special Issue entitled "Myocyte-Fibroblast Signalling in Myocardium."

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1. Heterocellular coupling between cardiac cells *in vivo*?

When we consider the cellular basis of cardiac function, we tend to focus on myocytes, 'forgetting' the seemingly silent majority of electrically non-excitable cells. These include endothelial, fat, immune and stem cells, but the largest sub-population is formed by connective tissue cells (fibroblasts). Non-excitable does not mean 'not exciting', however, as these cells are crucial for structural, biochemical, and electro-mechanical integrity of the heart [1–3].

1.1. 'The' cardiac fibroblast?

Until the 1990s, cardiac fibroblasts were largely considered to be of limited relevance beyond structural support (a bit like the traditional view on neuroglia). That changed with the discovery of fibroblast-mediated signalling two decades ago, and led to a step-increase in the number of publications on cardiac fibroblasts from tens or fewer to hundreds per year (roughly 99% of all cardiac fibroblast papers currently listed on *Web of Science* have been published after 1990). As a result of this surge, fibroblasts are now accepted as key contributors to development, adaptation, and disease-related remodelling of the heart (*e.g.* [4–7]). At the same time, we are still far from having comprehensive insight into the roles of connective tissue in the heart. In part, this is caused by the fact that 'the' cardiac fibroblast does not exist.

Cardiac fibroblasts originate from at least three progenitor populations. Firstly, fibroblasts arise from endocardium *via* epithelial-to-mesenchymal transformation [8]. Secondly, retroviral lineage tracing showed that fibroblasts also originate from the pro-epicardium, a group of embryonic progenitor cells known to give rise to the epicardium [9]. Thirdly, cardiac fibroblasts derive from bone marrow in normal development and as a contributor to the homeostatic maintenance of adult myocardium [10–12], but also during post-injury scar formation [13]. Fibroblasts therefore constitute a heterogeneous and dynamic population of cells, whose origin, regulation, and function in health [14] and disease [15], including their role in repair, impact on cardiomyocyte proliferation [16] and, possibly, their transformation into cardiac muscle cells [17], pose exciting challenges of high clinical relevance. In addition to heterogeneity related to their origin, cardiac fibroblasts respond to activation, such as during myocardial infarction, thorough phenotype transformation into myofibroblasts, which are regarded by some as a distinct cell type altogether (for recent reviews see [18–21]).

This review is focussed on exploring the presence, *in vivo*, of electrophysiologically relevant heterocellular connections between cardiac myocytes and connective tissue cells. Given that (i) the developmental origin and (ii) the precise state of cell-activation are not usually known or reported, and since (iii) neither of these aspects is of primary concern for exploring the principal question as to whether or not there is *in vivo* heterocellular coupling in the heart between the excitable muscle and the non-excitable connective tissue cells, we will refer to the latter as 'fibroblasts', aware of the inherent limitations of such abbreviated terminology.

1.2. Properties of fibroblasts in tissue and in a dish

For roughly half a century, the presence of electrotonic coupling between cardiac fibroblasts and myocytes has been a well-established fact *in vitro*. Since the mid-1960s, the synchronization of spontaneous contractile activity in isolated cardiomyocytes, interconnected solely by fibroblasts, has been noted and characterised using time-lapse microscopy, microelectrode recordings, and dye transfer studies [22–24]. More recently, using linearly structured cell cultures [25–28], optical mapping of voltage sensitive dyes has shown that fibroblast inserts can electrotonically bridge gaps between groups of myocytes that are up to 300 μm apart [29].

This ability of fibroblasts to act as long-distance conductors benefits from their high membrane resistance and relatively low capacitance [30]. When considering numerical parameter ranges for fibroblast electrophysiological properties, it is imperative to appreciate pronounced differences between cells in *vivo* and *in vitro* (even prior to electrophysiological remodelling of fibroblasts in cell culture [31]).

Cardiac fibroblasts *in vivo* form large sheet-like extensions, often with additional irregular folds, and elongated cytoplasmic processes [32,33] (not just in mammals; see on-line movie S1 of [34] with data from fish). Careful electron microscopy (EM) based reconstruction of an individual fibroblast in rabbit sino-atrial node revealed that it formed a membrane juxtaposition with a neighbouring myocyte covering 720 μm^2 [33]. As this reconstruction excluded some of the more distant fibroblast extensions, total surface area of this cell will have been 1500 μm^2, or more.

In contrast, fibroblasts, freshly-isolated from healthy myocardium, are rounded cells with initial diameters of about 7–9 μm [31,35,36]. EM data showed that they lack not only the typical membrane extensions, but also folds or membrane invaginations that otherwise could increase surface area [36]. Therefore, considering their near-spherical cell shapes, one can calculate the surface membrane area of freshly isolated fibroblasts as 150–250 μm^2, an order of magnitude less than in tissue. A probable reason for this discrepancy is the fact that cardiac cell isolation protocols tend to involve a combination of enzymatic digestion (to destroy connective tissue bonds) and mechanical agitation (to disturb tissue integrity), with the effect that surviving fibroblasts are likely to be the truncated non-myocyte fragments that contain a nucleus [37].

Currently, there is no data on the cell size distribution of fibroblasts *in vivo*, so we don't know how typical a surface area of 1500 μm^2 is for fibroblasts in the heart. As a ball-park value, though, it is in keeping with the observation that fibroblast membrane resistances *in situ* (0.5–1 GΩ [38,39]) are generally about an order of magnitude lower than in freshly isolated cells [40,41]. It stands to reason that fibroblast membrane capacitances *in vivo* (hard to quantify by direct electrophysiological means in these extended and mutually interconnected cells) exceed those of freshly isolated cells (typically 6–10 pF in fibroblasts isolated from healthy myocardium [31,41]). Even if that were by an order of magnitude as well, which is not inconceivable, it would still render fibroblast capacitances small compared to cardiac myocytes (values for ventricular cardiomyocytes, isolated from healthy tissue, range from about 150 pF in rabbit and ferret to 300 pF in rat [42]).

Fibroblasts have a relatively depolarised membrane potential (usually between -10 and -50 mV; in tissue at the less negative end of this range), whether recorded using sharp electrodes *in situ* [38,39], or using single [43,44] and dual [38,40] patch clamp *in vitro*. While precise membrane potential measurements with these direct (but invasive) electrophysiological techniques are challenging in individual cells when cell- and seal-resistances are in a similar ball-park, the above potential range is also evident from observations based on an indirect assessment of biological reporter systems (*e.g.* by monitoring fibroblast effects on cultured cardiomyocytes [45–48]). Therefore, in addition to potentially supporting conduction, cardiac fibroblasts can depolarize resting,

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