ORGANOIDS

An in vitro model of myocardial infarction

Structural and functional changes following infarction in a human heart can be modelled with human cardiac organoids set in a hypoxic gradient and stimulated with the neurotransmitter noradrenaline.

Richard Mills and James Hudson

Myocardial infarction — which is caused by the blockage of the arteries supplying blood to the heart — limits the delivery of oxygenated blood to the myocardium. Damaged myocardium has an increased risk of arrhythmia and a reduced ability to pump blood, which can often lead to heart failure. During this process, the adrenergic (or sympathetic) nervous system produces noradrenaline in an attempt to restore cardiac output by augmenting myocardial contractility, which can exacerbate the damage to an already dysfunctional heart. Animal models of myocardial infarction are a valuable resource to study the progression of the disease and to find putative therapeutic targets, however the ability to test large numbers of potential therapies in animals is limited by labour-intensive surgical techniques, the requirement for large animal numbers owing to the variability of the functional-outcome data, and the time-consuming sectioning, staining and imaging of the histopathology assays. Furthermore, differences in genetics and physiology between the small-animal models and humans may in some cases limit the usefulness of the data for finding treatments or for modelling the progression of cardiac disease in humans[1].

Engineered human heart tissue, including microtissues and organoids, serve as models for studying toxicology[2], genetic[3] and environmental[4] diseases, cellular interactions[5], tissue regeneration[6] and tissue maturation[7]. Although such engineered tissue models can facilitate the screening and discovery of therapeutics for heart disease[8], models of the infarcted human heart that integrate environmental factors contributing to cardiovascular disease are lacking. Reporting in *Nature Biomedical Engineering*, Ying Mei, Tong Ye and colleagues now describe human cardiac organoids as myocardial-infarction models, and the use of the organoids to assess the cardiotoxic and therapeutic actions of clinically relevant drugs[9].

Mei and co-authors' organoid-culture system is based on spheroids ~300 μm in diameter, consisting of 50% human pluripotent-stem-cell-derived cardiomyocytes (hPSC-CMs) and 50% non-myocytes (with a 4:2:1 ratio of human ventricular cardiac fibroblasts (hCFs), human umbilical vein endothelial cells (HUVECs) and human adipose-derived mesenchymal stromal cells (hADSCs)). The organoids were exposed to a gradient of oxygen concentration rather than cultured wholly in a hypoxic environment, which instead led to the death of all the cardiomyocytes and to the generation of a fibrotic shell in the microtissue. Noradrenaline was added to the culture media to maintain or slightly increase the rate of contraction of the organoids, and to induce an apoptotic response at

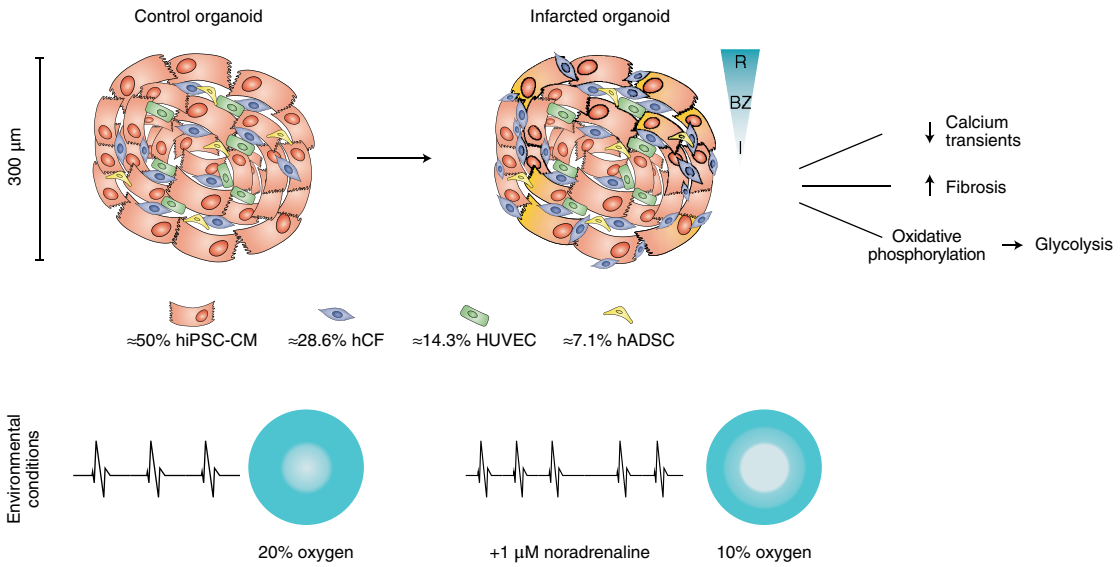

Fig. 1 | Human cardiac organoids as models of myocardial infarction. The cardiac organoids are composed of multiple self-organizing cell types: human induced-pluripotent-stem-cell-derived cardiomyocytes (hiPSC-CMs), hCFs, HUVECs and hADSCs. Environmental infarct conditions (10% oxygen and noradrenaline at 1 μM) led to the development of microtissues that resemble an infarct zone (I) at the centre, surrounded by a border zone (BZ) and by a remote fibrotic zone (R) on the outer surface. These changes result in decreased calcium transients, unsynchronized contractions and fibrosis, and in a shift from oxidative phosphorylation to glycolysis.

the organoid's centre (similar to what has been achieved with hypoxia induction in engineered mouse heart tissue[10]). Through carefully sized organoids, precise control over oxygen concentrations and the addition of noradrenaline, the authors created cardiac organoids with consistent 'infarction gradients' that structurally mimicked the zones of an infarcted human heart.

Mei and co-authors profiled the infarcted cardiac organoids via global gene-expression changes, showing that multiple differentially expressed genes overlapped with those seen in mouse and porcine myocardial-infarction models and in human ischaemic cardiomyopathy samples. The differentially expressed genes were reflective of ischaemic cardiac injury, including transcripts indicative of the gene-ontology terms 'response to oxidative stress', 'heart contraction' and 'fibroblast proliferation'. Principal-component analysis showed that the infarct signature was a minor contributor to the gene-expression variance (4.3%), with human-infarct-organoid samples and mouse myocardial injury data clustering separately. There was considerable transcriptomic variation between human and mouse species (accounting for 50.6% of the variance) and differences in tissue composition (accounting for 29.2% of the variance), suggesting that human-stem-cell-derived models and mouse models may differ in their downstream response to infarction. Also, genes expressed after ischaemic cardiomyopathy in patients and those expressed in human infarcted organoids were clustered apart, owing to differences in tissue complexity and maturation status, suggesting that human organoids also have limitations in their approximation to human physiological settings. Together, these results suggest that the organoid model of hypoxia-induced myocardial infarction recapitulated some pathology hallmarks of the disease, such as fibrosis and detrimental metabolic and functional changes.

Structural and functional analyses of the organoid infarction model revealed an increase in fibroblasts towards the outer edges, a higher proportion of myofibroblasts than in healthy organoids, and an increase in the expression of fibrotic markers. These changes underpinned increases in stiffness, reflective of changes that occur in fibrotic myocardium. Also, the infarcted organoids displayed a reduced oxygen-consumption rate, reduced oxidative phosphorylation, and increased glycolysis and lactate production, recapitulating the increased anaerobic metabolism that occurs during ischaemia in human infarcted myocardium in vivo. Moreover, by using two-photon scanned light-sheet microscopy to visualize calcium handling in the organoid interiors, Mei and colleagues observed reduced calcium-transient amplitudes and unsynchronized transients in the remaining cardiomyocytes at the centre of the infarcted organoids; in healthy organoids, interconnected cardiomyocytes instead beat in sync. They also show that the non-cardiac cell populations in the infarcted organoids played a key role in arrhythmia, as hPSC-CM-only microtissues (that is, containing no fibroblasts) retained coordinated calcium transients. These observations are consistent with processes typical of myocardial infarction in vivo, as well as with the decreased expression of calcium-handling proteins, cardiomyocyte death and fibrosis (Fig. 1).

Mei and co-authors used the organoid model to assess the responses of the infarcted organoids to clinically relevant drugs. For the anticancer drug doxorubicin (which can cause heart failure of varying severity), they show that the infarcted organoids displayed a more severe fibrotic response at a lower doxorubicin concentration than healthy controls, potentially highlighting environmental conditions that exacerbate doxorubicin toxicity. They also show that JQ1, a bromodomain and extra terminal domain 4 (BRD4) inhibitor and putative therapeutic for cardiac fibrosis[11], prevented fibroblast proliferation and reduced unsynchronized contractions in the infarcted organoids. This is relevant because arrhythmia is a major cause of death in patients who have had a myocardial infarction[12].

Differences between the in vitro infarcted organoids and ischaemic cardiomyopathy in vivo may be explained by the in vivo response to infarction and the differentiation and maturation stages of all cell types (in particular non-cardiomyocytes, which segregate into a wide array of phenotypes and activation states[13]). As cell composition and phenotype play key roles in the progression of infarction and in clinical outcomes, recapitulating cell-maturation state and cellular complexity in the organoids may be integral to their degree of usefulness for drug discovery and disease modelling. The limited complexity of the organoids is also reflected in their lacking of cells from the immune system, which are major modulators of myocardial-infarction pathology[14]; therefore, inclusion of other cell types may also be needed. Furthermore, including the effects of reperfusion injury (the tissue damage caused when the blood flow is restored to previously ischaemic tissues) such as the increase in succinate and the production of reactive oxygen species and cell death could improve the capabilities of the model in predicting drug efficacy.

Sophisticated culture systems featuring three-dimensional environments, the integration of multiple cell types and their interactions, the inclusion of mechanical loading and specific environmental culture conditions are required to recapitulate the essential in vivo structural and functional characteristics of a range of tissues. Mei and colleagues' infarcted organoids are a step towards a more realistic model of myocardial infarction for the screening, identification and validation of therapeutics for cardiac disease. ❐

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Competing interests

R.M. and J.H. have collaborated with AstraZeneca for the discovery of new heart-failure drugs, are on the scientific advisory board of Dynomics for the discovery of new heart-failure drugs, and have patents related to bioengineered-tissue technologies and culture conditions, and to putative therapeutic targets for heart failure.