Evaluation of a new media designed to drive improved maturity of iPSC-derived cardiomyocytes for cardiac disease, cardiotoxicity and drug screening

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Abstract

Despite decades of effort, there is a pressing need for better *in vitro* cardiac and cardiotoxicity models. Cardiovascular diseases (CVD) remain the leading global cause of death and cardiotoxicity is responsible for a third of all pre-clinical pharmaceutical regulatory failures. While traditional models (*ex vivo* and *in vivo* animal models, primary human tissue and immortalised cell lines) have provided valuable insights, there remains a translational gap between the "bench and bedside" due to issues with human-relevance, alongside well-characterized challenges with availability, throughput and cost.

Researchers have therefore been turning to human iPSC-derived cardiomyocytes for their potential to provide a limitless source of consistent, human-relevant cardiac cells. By taking donor cells, reprogramming them to an iPSC state and then differentiating them into cardiomyocytes, researchers can mimic human physiology in a scalable format, using these cells to power advanced *in vitro* cardiac disease and cardiotoxicity models.

However, standard differentiation protocols can lead to less mature cardiomyocyte phenotypes, impairing functional performance and therefore the utility of human iPSC-based models. Key measures of immature phenotype include the following parameters: poor sarcomere alignment, low cardiac maturity marker expression, greater levels of spontaneous beating, longer field potential duration (FPD) and slower conduction velocity (CV).

Axol Bioscience, in collaboration with partner organizations, have been exploring a new metabolic maturation media to enhance the maturity of human iPSC-derived cardiomyocytes, enabling better models for cardiac research, disease and cardiotoxicity. This media is referred to as 'cardiac maturation media' in this poster.

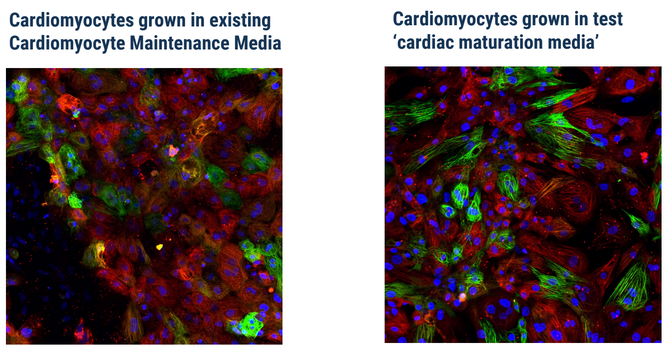

Figure 1. iPSC-derived cardiomyocytes differentiated and maintained in the current axoCells™ Cardiomyocyte Maintenance Media can exhibit a less mature phenotype. Axol is exploring a cardiac maturation media which seeks to improve these indicators of maturation, helping to produce more robust cardiac models.

5 indicators of improved cardiomyocyte maturity	
1	**Improved expression of cardiac maturity markers**
2	**Well-structured sarcomere network**
3	**Reduced spontaneous beating**
4	**Electrophysiology- shorter field potential duration**
5	**Electrophysiology- faster conduction velocity**

The challenge with *in vitro* cardiac models

One of the significant hurdles to the wider adoption of human iPSC-derived cardiomyocytes (iPSC-CMs) as a physiologically relevant, non-animal cardiac model is that iPSC-CMs differentiated maintained in traditional cardiac maintenance media such as axoCells™ Cardiomyocyte Maintenance Media (ax2530) tend to exhibit a markedly immature phenotype. Key indicators such as morphology, marker expression, calcium handling, electrophysiology and contractility all reflect an immature phenotype undermining the physiological relevance of iPSC-CMs to correctly model the cardiac function, disease, toxicology and pharmacology of adult human hearts.

Therefore, Axol has collaborated with researchers at the University of Toronto† to explore a novel media that we developed using an iterative algorithm with the aims of driving a more mature phenotype (see Callaghan *et al.* 2022, QR link below).

Here Axol demonstrates the improvement in maturity markers seen in two of our iPSC-CMs lines ax2508 and ax5858 against the criteria outlined in **Figure 1** when using this media.

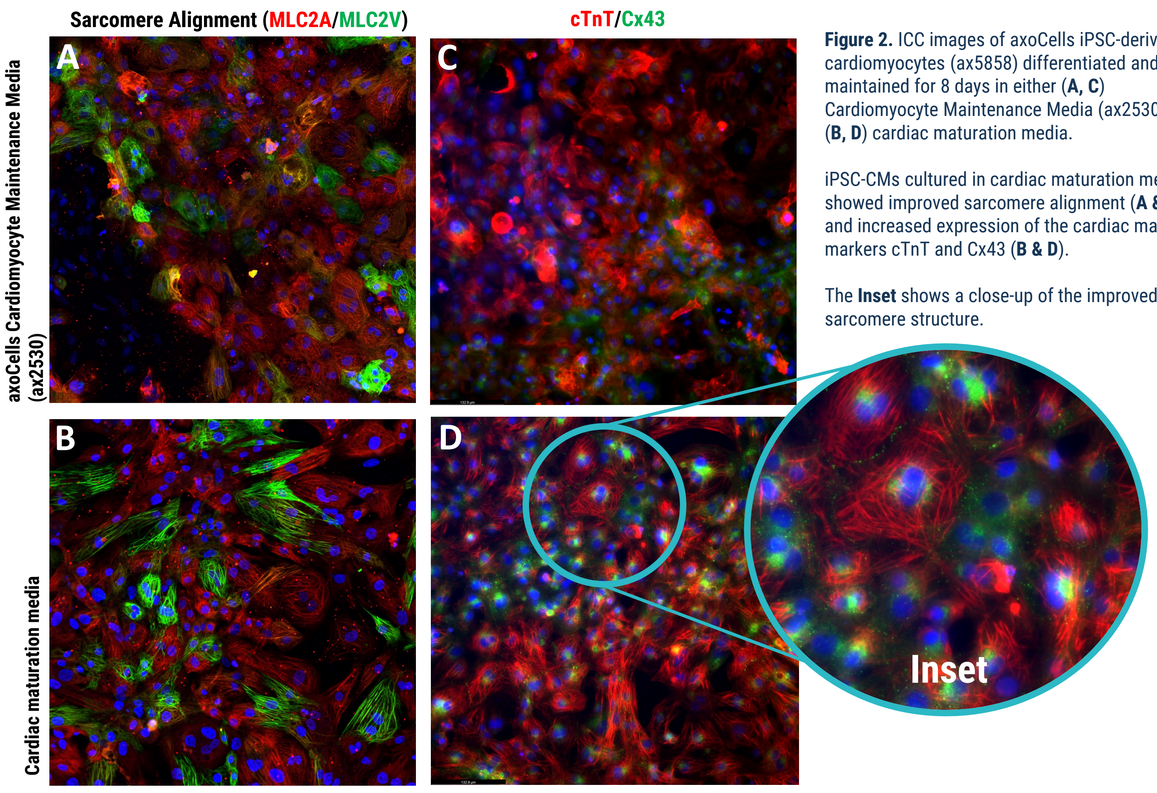

Figure 2. ICC images of axoCells iPSC-derived cardiomyocytes (ax5858) differentiated and maintained for 8 days in either (**A, C**) Cardiomyocyte Maintenance Media (ax2530) or (**B, D**) cardiac maturation media.

iPSC-CMs cultured in cardiac maturation media showed improved sarcomere alignment (**A & B**) and increased expression of the cardiac maturity markers cTnT and Cx43 (**B & D**).

The **Inset** shows a close-up of the improved sarcomere structure.

ICC imaging demonstrates improved maturity with the cardiac maturation media

axoCells iPSC-derived cardiomyocytes (ax5858) were cultured to Day 8 post-thaw in either Cardiomyocyte Maintenance Media (ax2530) or the cardiac maturation media. By Day 8 the iPSC-CMs cultured in cardiac maturation media displayed improved sarcomere alignment (**Figure 2B & Inset**) and improved expression of the maturity markers cardiac troponin (**cTnT**) and Connexin 43 (**Cx43**) (**Figure 2C**). Improvements in **Cx43** expression may also explain the increases seen in Conduction Velocity with the cardiac maturation media too (**Figure 4B.**)

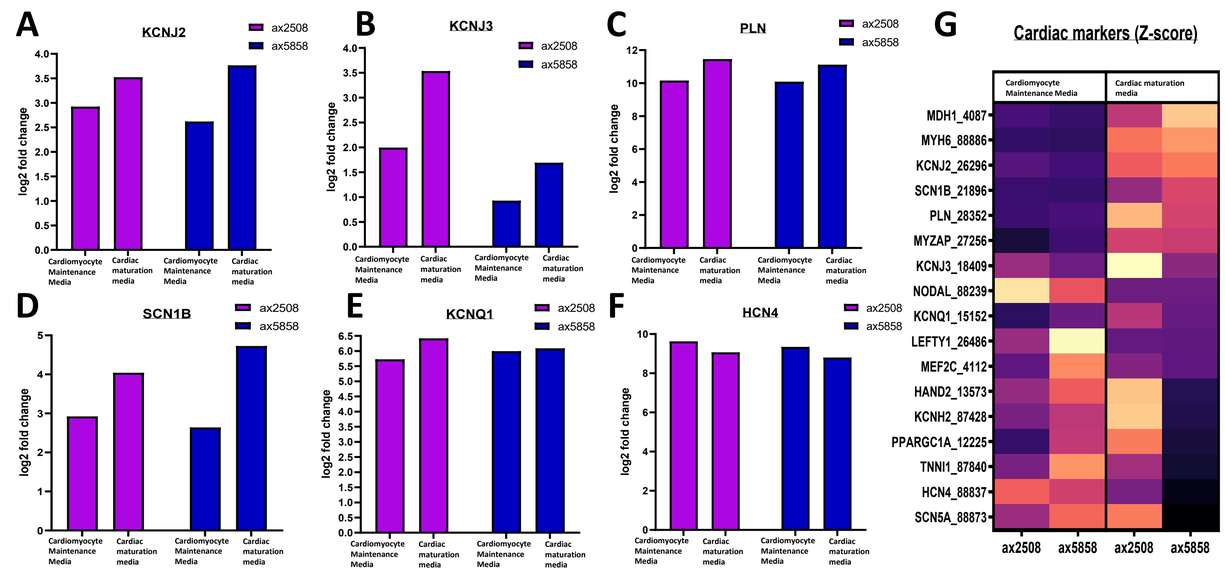

Figure 3. Comparison of TempO-Seq Expression levels (at DIV8) of key maturation markers in two of axoCells ventricular iPSC-CMs (ax2508 and ax5858) cultured in the Cardiomyocyte Maintenance Media and the cardiac maturation media. Increased expression levels seen in both lines for the maturity markers, KCNJ2 (**A**), KCNJ3 (**B**), PLN (**C**), SCN1B (**D**) and KCNQ1 (**E**) with a decrease in levels of the pacemaker channel HCN4 (**F**). The Z score for maturity markers (**G**) broadly supports these findings with some cell-line-specific differences.

TempO-Seq expression levels of key maturity markers indicate greater maturity of iPSC-CMs from two lines cultured in cardiac maturation media

Examination of key maturity markers reveals significant changes in the levels seen in both iPSC-CM lines (ax2508 & ax5858) cultured in the cardiac maturation media indicating greater maturity. Markers of immaturity such as MEFC, NODAL, LEFTY and TNNI1 (**Figure 3G**) are reduced in both cell lines as is the pacemaker channel HCN4 (**Figure 3F**). In contrast, important markers such as KCJN2 (**Figure 3A**), PLN (**Figure 3C**), SCN1B (**Figure 3D**) and KCNQ1 (**Figure 3E**), which are only present in low levels in immature iPSC-CMs have had their levels markedly increased by the maturation media for both cell lines. Similarly, KCNJ3 which had noticeably low levels in the immature cardiomyocytes cultured in Cardiomyocyte Maintenance Media has also significantly increased in both iPSC-CM cell lines (**Figure 3B**). Although the relative decrease in immaturity markers and increase in maturity markers is broadly seen in both iPSC-CM cell lines, there are some cell-line specific differences too, such as mixed effects for KCNH2, PPARG, SCN5A and HAND2 (**Figure 3G**).

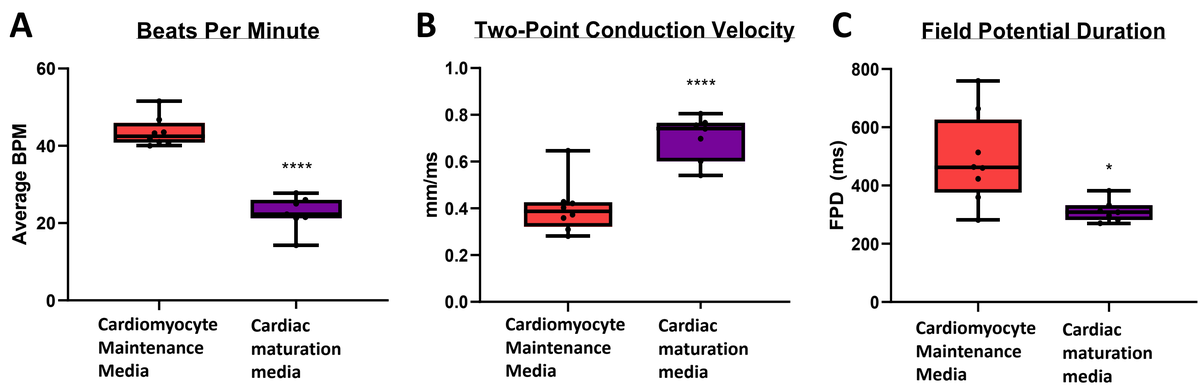

Figure 4. Comparison of key MEA parameters (at DIV14) from axoCells ventricular iPSC-CMs (ax5858) cultured in either Cardiomyocyte Maintenance Media or the cardiac maturation media. Reduced Beat Rate (**A**) and Field Potential duration (**C**) and increased Conduction velocity (**B**) are all indicative of increased maturity of iPSC-CM function.

Functional MEA data replicates findings seen with ICC and Transcriptomics

In support of the observed reduction in the pacemaker HCN4 expression (**Figure 3F**) and increased expression of Connexin 43 (**Figure 2C**), functional data obtained using an Axion MEA Maestro Pro™ showed a significant reduction in spontaneous Beat Rate (**Figure 4A**) and an increase in Two-Point Conduction Velocity (**Figure 4B**) indicating a more mature functional phenotype. Similarly, the typically lengthy FPD seen in immature iPSC-CM was also significantly reduced by the cardiac maturation media (**Figure 4C**), further evidence of functional maturity and allowing a better, more physiological baseline for pro-arrhythmia compound toxicology screening, for example.

Conclusion

Existing iPSC-based models can be limited by the maturity of the cells that fuel them, with the cell's apparent 'immaturity' reducing the utility of the models. The cardiac maturation media produces more phenotypically mature iPSC-derived cardiomyocytes than traditional cardiac maintenance media across all five parameters, tackling one of the major obstacles to the wider adoption of iPSC-derived cardiomyocytes.

By enhancing maturity, the cardiac maturation media looks to improve the functional performance of iPSC-derived cardiomyocytes which can then be used to build better models for cardiac research, drug discovery and cardiotoxicity screening. Future work will look to widen the scope of maturity markers and focus on developing this media to produce an early-access product for testing.

References

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† Cardiac maturation media has been licensed from the University of Toronto (USA patent application no. 63/280,388)

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