Electroconductive Hydrogel Scaffolds to Enhance Maturation of Human iPSC-derived Cardiac Tissues

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Introduction: Heart failure is the leading cause of mortality worldwide. Injectable hydrogels or implantable cardiac patches have demonstrated tremendous promise for treatment of injured heart tissue upon myocardial infarction (MI). In this regard, nanoengineered electroconductive biomaterials have shown tremendous promises to improve function and maturation of cardiomyocytes (CMs). However, the mechanism by which these nanomaterials contribute to improved function and maturation of cardiac tissues has not been fully investigated yet. In addition, most of the previous works have mainly utilized neonatal rat ventricular CMs rather than human stem-cell derived CMs, (i.e., hiPSC-CMs) to conduct their studies. In this study, we investigated the influence of gelatin methacryloyl (GelMA)-incorporated gold nanorods (GNRs) hydrogels on maturation of hiPSC-CMs. To dissect the underlying mechanism, non-conductive silica nanoparticles (SNPs), with similar sizes to GNRs, were incorporated into GelMA hydrogels, as a control for electroconductivity and bio-physical cues, responsible for enhanced maturation of the cells.

Materials and Methods: hiPSC-CMs and hiPSC- Cardiac Fibroblasts (hiPSC-FBs) were differentiated from IMR90-4 stem cells (WiCell) as described elsewhere.[1] GNRs and GelMA were synthesized using customized protocols as described previously[2] and SNPs were purchased commercially (GEANDR-Co.). The formation and purity of GNRs were confirmed by TEM and the lyophilized hydrogels were imaged via SEM. Four groups of hydrogel scaffolds including GelMA-Pristine(15%w/v), GelMA-GNR (1.0mg/mL) (**G-GNR**), GelMA-SNP(1.0mg/mL) (**G-SNP1**), and GelMA-SNP(1.4mg/mL) (**G-SNP1.4**; maintaining similar numbers of SNPs to 1.0 mg of GNRs) were synthesized. The electroconductivity of the hydrogel scaffolds was analyzed by impedance measurement at various frequencies using EIS Potentiostat. hiPSC-CMs were co-cultured with hiPSC-FBs (7:1 ratio) on top of the hydrogel substrates for 14 days. Immunofluorescence (IF) staining was performed to stain sarcomere α-actinin (SAA), connexin-43 (Cx-43) and nuclei. qPCR analysis was carried out with iTaq Universal SYBR Green Supermix (BioRad) with 18S as the housekeeping gene and the plates were analyzed with qTower 2.0, to assess upregulation of specific genes as function of nanomaterials.

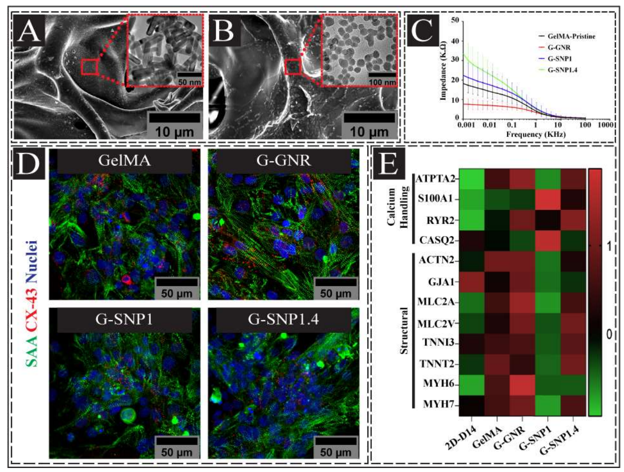

Figure 1. (A&B) SEM images of the G-GNR and G-SNP1 hydrogels. The subsets are the TEM images of GNRs and SNPs. **(C)** The Bode diagram, showing that the impedance of G-GNR was lower than that of GelMA, G-SNP1, and G-SNP1.4 hydrogels (n=6). **(D)** Images of the expression of cardiac-specific markers (SAA (green) and Cx-43 (red)) in the hiPSC-CMs cultured on the GelMA, G-GNR, G-SNP1, and G-SNP1.4 substrates. Nuclei were stained with DAPI (blue). **(E)** Gene expression data for hydrogel groups and aged-match condition (n=3).

Results and Discussion: TEM imaging showed the formation of highly purified GNRs with an average length of 42.7nm ± 4.8 (mean ± S.D). The SEM images confirmed the successfull incorporation of GNRs and SNPs into the GelMA hydrogel (**Fig. 1A&B**). The Bode diagram illustrates reduced impedance of the G-GNR compared to other hydrogel scaffolds suggesting its enhanced electroconductivity (**Fig. 1C**). IF images confirmed the expression of striated SAA and Cx-43 proteins in hiPSC-CMs cultured on G-GNR hydrogel and to a lesser extent in non-conductive GelMA and G-SNP hydrogels, suggesting that GNRs positively impacted the formation of more mature cardiac tissues (**Fig. 1D**). The gene expression heatmap demonstrating enhanced upregulation of structural and calcium handling genes across the hydrogels especially on G-GNR as compared to 2D monolayer (**Fig. 1E**).

Conclusion: Our data suggests that GNRs distributed throughout GelMA hydrogels positively induced the formation of more mature cardiac tissues as evident by abundance of striated SAA and higher expression of Cx-43. This improvement was also confirmed by upregulation of calcium and structural handling genes.

References: **1**. Veldhuizen, J. et al. Biomaterials **2020**, 256 (120195). **2**. Navaei, A. et al. Acta Biomaterialia **2016**, 41(133-146). **Acknowledgments:** This project was supported by the NSF Award # 2016501.