# Differential Gene Expression in C2C12 Cells due to Scaffold Structure-Property-Processing-Performance Correlations

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**INTRODUCTION:** Graphene – an atomically thin layer of carbon atoms arranged in a hexagonal lattice – has gained interest as a bioscaffold for tissue engineering for its exceptional mechanical, electrical, and thermal properties[1-5]. Graphene's structure and properties– tightly coupled to synthesis and processing conditions[4]– are thought to influence biomolecular interactions at the graphene-cell interfaces. In this study, C2C12 cells, a multipotent mouse myoblast cell line, were grown on graphene bioscaffolds with specific structure–property–processing–performance (SP3) correlations. We found that SP3 correlations significantly influenced C2C12 differentiation, myotube formation, and gene expression, suggesting that graphene–cell interfaces can be engineered to control biomolecule structure and function in adherent cells. The experiments performed are the first to make a direct comparison on scaffold SP3 correlations and their impact on genetic expression of multipotent myoblast cells.

**METHODS:** Chemical vapor deposition (CVD) grown graphene film, transferred to quartz glass slides, resulted with surface roughness (Ra) of 2.37 nm and graphene ink– produced by solvent assisted exfoliation– was printed using a Dimatix inkjet printer onto glass coverslips (Ra of 10.0 nm). Epitaxial graphene on SiC wafers, produced by Si sublimation (Istituto Italiano de Tecnologia, Pisa, Italy) (Ra of 2.24 nm). Prior to cell integration, cell attachment was facilitated with agarose gel applied to the edges of all graphene bioscaffolds and corresponding control bioscaffolds (quartz glass, glass coverslips and SiC wafers). C2C12 cells, maintained in a humidified incubator at 37°C and 5% $CO_2$, were seeded onto the scaffolds at 1.3 x $10^4$ cells/$cm^2$ in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). Cell growth and proliferation were monitored by transmitted light microscopy with images captured to study cell morphology. Gene expression patterns for each bioscaffold were analyzed for muscle myogenesis via qRT-PCR using a PCR array for muscle myogenesis and myopathy.

**RESULTS:** Preliminary results show differences in cell morphology varied in a manner dependent upon the specific bioscaffold used. Myotube-like structures were evident in the control, CVD and epitaxial graphene (Fig.1A). Each of the three graphene bioscaffolds resulted in a unique overall genetic profile related to muscle myogenesis. However, four up-regulated genes were common to all biosaffolds when compared to their control. These genes are as follows: dystrophin (Dmd), myostatin (Mstn), leptin (Lep), pyruvate dehydrogenase kinase isoenzyme 4 (Pdk4) and Utrophin (Utm) (Fig. 1B).

**DISCUSSION:** This preliminary work successfully shows that epitaxial, CVD, and printed graphene bioscaffold's SP3 correlations impact the gene expression of C2C12 cells in culture. Each graphene bioscaffold presented with different and unique muscle gene expression profiles when compared to the control. The different morphologies of the cells as viewed in the transmitted light microscopy images confirms that the C2C12 cells grown on each bioscaffold, are uniquely different than the control. The following biomarkers: dystrophin, myostatin, leptin, pyruvate dehydrogenase kinase isoenzyme 4, and Utrophin were all upregulated in each of the graphene bioscaffolds. Epitaxial graphene shows the most genes upregulated that correlate to the muscle genotype followed by printed graphene and CVD graphene which showed the least number of upregulated genes relating to muscle (Fig. 1C).

**REFERENCES**: 1. Nayak et al., ACS Nano., 2011. 2. Li et al., Sci Rep., 2013. 3. Krueger et al., ACS Biomaterials Science & Engineering, 2016. 4. Novoselov et al., Nature, (2012): 192-200. 5. Nieto et al., Advanced Functional Materials, 2015.

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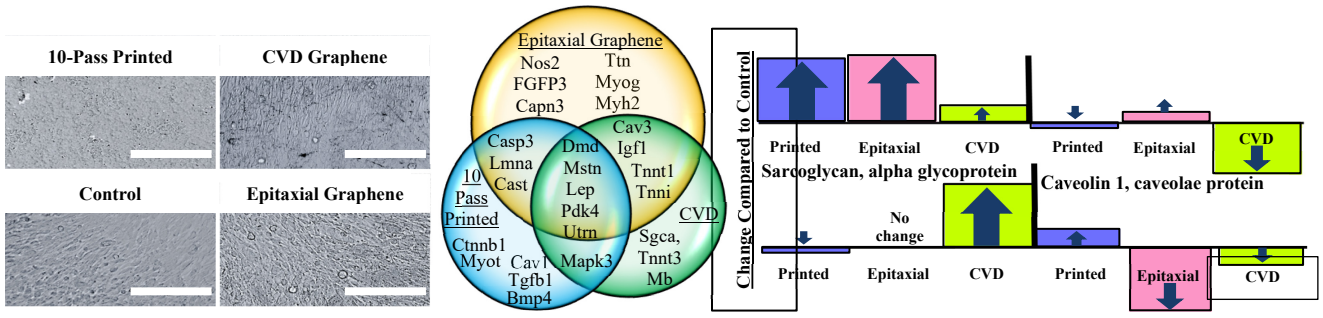


**Figure 1.** C2C12 cells on the graphene bioscaffolds. **A)** Transmitted light images of C2C12 cells in DMEM 10% FBS 1% P/S media of all graphene bioscaffolds and the control for 7-day time-points. Scale bar 200 µm . B) Muscle gene profiles for epitaxial graphene, 10-pass printed graphene and CVD when compared to the control. **C)** The up-regulation and down-regulation of genes found in muscle cell membrane compared to the control.