3D-Printed High-Content Graphene Composites for Tissue Engineering

Ramille N. Shah, Adam E. Jakus, Alexandra L. Rutz, Sumanas W. Jordan
Simpson Querrey Institute for BioNanotechnology, Materials Science & Engineering, Surgery; Northwestern University

Statement of Purpose: Graphene, or single sheet graphite (pure carbon), as a biomaterial has received increased interest in recent years due to its high conductivity, mechanical robustness, and potential biocompatibility. Numerous studies have shown that it has significant potential in the field of tissue engineering. It has also been shown to be an excellent candidate for bioelectronics, biosensors, and drug delivery applications. However, due to its nanoscopic nature, macroscopic, larger-scale majority graphene objects have not been able to be fabricated until now. Here, we present the first case of a majority graphene (3DG) composite, 3D-printed (3DP) from liquid inks, which displays unique electrical, mechanical, and biological properties.

Methods: Graphene 3D inks of various concentrations were synthesized via suspension, dissolution, and agitations of 20, 40, and 60 (AKA 3DG) vol.% (solids: polymer + powder) graphene powders in solutions containing 80, 60, or 40 vol.% (relative to powder) polylactide-co-glycolide (PLG) in a graded solvent mixture. The inks were printed into 3D constructs for electrical, mechanical, *in vitro*, and *in vivo* testing using a 3D-Bioplotter (EnvisionTEC, GmbH), a cartridge and extrusion-based 3D printing platform. The microstructure of 3D-printed constructs were investigated using scanning electron microscopy (SEM). Quasi-static compressive and tensile mechanical testing were performed on 3D-printed cylindrical and "dog-bone" specimens, respectively. Electrical resistivity measurements were performed using a 4-point probe setup. *In vitro* experiments were performed by seeding human mesenchymal stem cells (hMSCs), iPS cell-derived human neurons (iNeurons), and mouse cardiomyocytes onto washed and sterilized 3D-printed, porous scaffolds and cultured for up to 3 weeks. Confocal fluorescent and SEM imaging were used to observe cell viability and morphology, in addition to DNA quantification and gene expression analysis through qPCR. *In vivo* biocompatibility experiments were performed on female BALB/c mice using a subcutaneous (SubQ) implant model using 60 vol.% graphene and pure PLG scaffolds as controls. Scaffolds and surrounding tissue were removed 7 and 30 days after implantation and were analyzed using histological staining and imaging as well as electron-histological SEM techniques.

Results: Our novel high content graphene inks can be used to 3D-print highly precise, relevant-scale 3DG constructs (**1A**). With a brief exposure to 100˚C temperatures, 3DG's conductivity can increase two-fold to ~800 S/m, the highest ever recorded for a non-metallic 3DP material (**1B**). Mechanical testing revealed that 3DG can be tensile strained to ~60%, while 20 vol.% graphene can be strained upwards of 200% without failure. Similar behavior is observed under compression. 3DG, despite being primarily graphene, displays plastic mechanical properties: thin sheets can even be cut, folded, and rolled (**1C**). hMSCs culture in simple DMEM proliferation medium are not only highly viable and proliferate rapidly on and within the various graphene composites (**1D**), but also develop very distinct, elongated, morphologies (**1E**) after only a few days that are not observed on PLG controls. *In vivo*, surrounding SubQ tissue rapidly integrates into 3DG scaffolds (**1F,G**) over the course of 30 days, which also becomes highly vascularized. Stains for COX-2 and neutrophils came up negative for both 7 and 30 day time points. However, macrophages were present in significant numbers at day 30 and appeared to be physically stripping graphene flakes off of the bulk material (**1G**, inset), which was further observed and characterized using SEM imaging (**1H**).

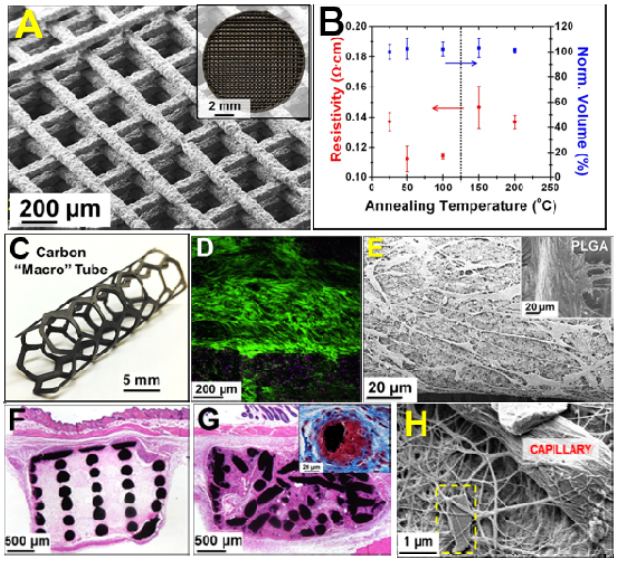

Figure 1. (**A**) SEM micrograph of 3DG cylinder (inset) structure. (**B**) Electrical resistivity of 3DG as a function of annealing temperature. (**C**) Example of the flexibility and formability of 3DG. (**D**) Live (green)-Dead (red) fluorescent scanning confocal reconstruction of hMSCs on 3DG 21 days after seeding. (**E**) SEM micrograph of hMSCs on 3DG strut after 7 days. (**F,G**) H&E histological images of 3DG subQ samples 7 and 30 days after explantation, respectively. Inset in **G** is Masson's trichrome illustrating macrophages concentrated around 3DG material. (**H**) SEM micrograph showing graphene flake within tissue in day 30 explanted sample.

Conclusions: Using a solution-based, scalable ink, graphene can be 3D-printed under ambient conditions with filaments ranging in diameter from 100 to 1000 µm. This particular composite system, comprised primarily of graphene, has advantageous mechanical, electrical, and biological properties. Not only does 3DG display the highest electrical conductivity of any carbon-based 3D-printed material reported to date, it is also mechanically flexible, has promising biocompatibility, and is biodegradable. These initial studies indicate that 3DG and its variants may be an incredibly promising new 3D-printable biomaterial worthy of additional functional studies, including those relating to cardiac, muscle, and spinal cord regeneration, as well as bioelectronics and biosensors.