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Extrusion printed graphene/polycaprolactone/ composites for tissue engineering

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Keywords

extrusion, tissue, printed, engineering, graphene, polycaprolactone, composites

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Extrusion printed graphene/ polycaprolactone/ composites for tissue engineering

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Keywords: Polycaprolactone, graphene, nanocomposites, biocompatible, melt extrusion printing, additive fabrication

Abstract. In this work fibres and complex three-dimensional scaffolds of a covalently linked graphene-polycaprolactone composite were successfully extruded and printed using a melt extrusion printing system. Fibres with varying diameters and morphologies, as well as complex scaffolds were fabricated using an additive fabrication approach and were characterized. It was found that the addition of graphene improves the mechanical properties of the fibres by over 50% and *in vitro* cytotoxicity tests showed good biocompatibility indicating a promising material for tissue engineering applications.

1. INTRODUCTION

Functional synthetic biomaterials, especially biopolymers and composites, have recently been the focus of intense research efforts [**Error! Reference source not found.**]. One of the most important applications of these new biomaterials is the development of scaffolds for tissue engineering. In tissue engineering, cells are seeded or grown onto a biodegradable polymer scaffold to promote the growth and remodelling of tissue. In forming the new tissue, the biodegradable scaffold degrades at a controlled rate and is absorbed or excreted by the body. There have been extensive studies on the tissue engineering applications of synthetic and biodegradable polymer scaffolds in the laboratory and clinic [**Error! Reference source not found.**]. Polycaprolactone (PCl), for example, is an aliphatic polyester, that has attracted a lot of interest for a wide variety of biomedical and material applications due to its biocompability, biodegradability, mechanical properties and facile, cost-effective fabrication methods [2-6].

To further improve the properties of biocompatible materials, biopolymers have been used as a matrix for the incorporation of suitable fillers [**Error! Reference source not found.**]. Graphene is a single layer two-dimensional atomic sheet of carbon atoms exhibiting unique mechanical, electrical, optical, thermal and magnetic properties and has recently been shown to be biocompatible and advantageous for the growth of cells [**Error! Reference source not found.**-[10]], making it an excellent candidate for use as filler in polymer composites. Recently a number of groups have shown promising improvements in the mechanical, thermal and electrical properties of biopolymers using graphene

and graphene oxide biocomposites [6, **Error! Reference source not found.-Error! Reference source not found.**]

In our previous work [**Error! Reference source not found.**, [16]] we introduced covalently linked chemically reduced graphene /polycaprolactone composites which showed large improvements in the conductivity and mechanical properties of the polymer. Initial *in vitro* cytotoxicity testing also showed good biocompatibility and analysis of the degradation rates showed consistent, non-toxic biodegradation of the composite material [**Error! Reference source not found.**]. Importantly, the melt properties of the polymer were also retained on the addition of graphene resulting in an easily processable material ideal for fabrication into tissue engineering scaffolds using fused deposition modelling (FDM).

The fabrication of scaffolds using printing techniques is a relatively new method that promises to overcome the limitations of conventional scaffold fabrication methods, such as producing an interconnected structure for ingrowth of cells or controlling the pore size for cell migration and diffusion [**Error! Reference source not found.**]. Park et al [[18]] fabricated polycaprolactone scaffolds with interconnecting pores using a 3-D melt plotting system and compared the plotted scaffolds to those made by salt leaching. Based on their findings, the plotted scaffold was more suitable for cell ingrowth than a salt-leached scaffold. Lee et al [[19]] fabricated a 3D scaffold using similar rapid prototyping (RP) technology for tissue engineering and characterized the influence of pore geometry on the mechanical properties of the strand.

In this paper, covalently-linked graphene/polycaprolactone composites are extruded/printed in the form of fibres and scaffolds for the first time. The mechanical properties and biocompability of the extruded fibres are investigated to study the effect of graphene content.

2. MATERIALS AND METHODS

2.1. Preparation of graphene/Polycaprolactone extruded fibres and printed scaffolds

Covalently-linked graphene/polycaprolactone composites (PCl-G) with different graphene contents were synthesized following the method described in our previous work [**Error! Reference source not found.**]. In short, graphene nanosheets well dispersed in N,N'-dimethylformamide at a concentration of 0.5 mg/m[20]] were covalently modified with polycaprolactone (MW 80,000) using N,N'-dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP). The fibres were extruded using a KIMM SPS1000 bioplotter extrusion printing system (Fig. 1), in which the extrusion assembly is mounted on a three-axis stepping system above a level stage for full mechanised control of its XYZ axes via an associated computer system with customised software. Materials are extruded through interchangeable tips with internal diameters of 100-500 μm attached to a pressurised stainless steel barrel enclosed in a temperature controlled jacket. This printing assembly allows for great flexibility of parameter control due to its control of pressure, melt temperature and nozzle diameter. For extrusion of these composite materials, the barrel temperature was set to 125^{o}C and the applied pressure varied between 250-500 kPa. To obtain simple, rounded fibres printing was done without a substrate. To obtain more complex 3-D scaffolds a layer-by-layer, additive fabrication approach onto a glass substrate was used.

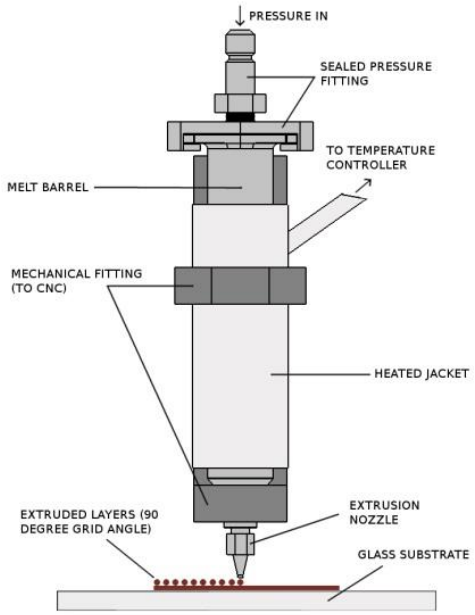

Fig. 1. Schematic of KIMM SPS1000 bioplotter extrusion printing system.

2.2. Characterization

Scanning electron microscopy (SEM) images were collected with a field-emission SEM instrument (JEOL JSM-7500FA). Differential scanning calorimetric (DSC) analysis was performed on a DSC Q100, TA Instruments. Mechanical properties were tested using an Instron 5566 Universal Testing Machine. To prepare samples for mechanical properties tests, the fibres were cut to the length of 10 mm and the thickness of the samples was measured using scanning electron microscopy (SEM) and fibres with 300-365 µm were used. The mean and standard deviation was reported for n = 5. The complex viscosity of the composites was determined using dynamic frequency sweep tests above the melting point of the composites on a TA instruments AR-G2 rheometer. Biocompatibility was tested using electroactive rat pheochromocytoma cells (cell line PC-12) grown on the extruded materials after preparation of materials (sterilisation with 70% ethanol and soaking in Dulbecco's modified eagle medium (DMEM) overnight). 1 mL of media (DMEM + 10% horse serum + 5% fetal bovine serum) containing 20,000 cells was placed into each well, and the cells were allowed to settle for 24 hours, before the media was changed to differentiation media (DMEM + 2% horse serum + 50 ng/mL nerve growth factor (NGF)). The cells were incubated at 37 ^{o}C in 5% CO_2 for 5 days before fixation with paraformaldehyde, staining with phalloidin-Alexa 488 (Invitrogen) and 4',6-diamidino-2-phenylindole (DAPI), and microscopy.

3. Results and Discussions

Covalently-linked graphene/polycaprolacone composites have been shown to be both electrically conductive and biodegradable and to have superior physical and electronic properties to binary mixtures of the two components [**Error! Reference source not found.**]. This is due to the homogeneous dispersion of graphene throughout the polymer matrix, resulting in less agglomeration of graphene nanosheets. The composites retain some of the mechanical strength and conductivity of graphene but also the flexibility, biocompatibility and, importantly for fabrication, the solubility and melt

properties of the polymer matrix. Due to the low melt temperature of polycaprolactone (~ 60 °C), the covalently-linked composites could be controllably processed into a variety of 2 and 3-dimensional structures similar to that used for tissue engineering using hot melt extrusion printing.

3.1. Melt behaviour

Melt extrusion printing is very dependent on a number of material parameters, especially the melt temperature and viscosity. Fig. 2 shows the change in the melting point and viscosity of the composites with increasing graphene content. Differential scanning calorimetry showed that the addition of graphene to polycaprolactone did not significantly affect the melting point of the composites, which remains between 55-60 °C. Graphene however, has an increasing effect on complex viscosity (η*) of the polymer composites. Graphene contents above 5% show an increase in the viscosity indicating the strengthening effect of graphene on PCl. However, the maximum viscosity of the synthesised composites was found to be 33560 Pa.s (5% graphene), which is well within the capabilities of the printer.

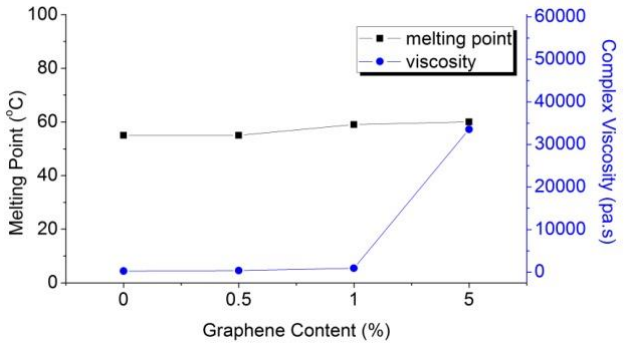

Fig. 2. Melting point and viscosity of PCl and graphene/PCl composites at different graphene contents.

3.4. Printing

The composites were printed/extruded successfully in the form of scaffolds and fibres with easily controllable diameters and fibre morphologies. The diameter of the extruded fibres could be controlled by either changing nozzle diameter or temperature. Applied pressure and/or head speed were varied between 100 and 500 μm while retaining fibre shape (Fig. 3). Fibres with rounded cross-sections were produced without using a substrate, instead extruded into free space and collected.

Complex three-dimensional scaffolds were printed onto a glass substrate. Fig. 3c shows a multi-layer lattice with a fibre thickness of 220 μm and a pore-size of 130 μm which is intended as a 3-dimensional cell scaffold. Fig. 3d shows a similarly sized scaffold with alternating conducting graphene containing and insulating non-graphene containing matched concentric circles which can be used to examine the effects of cell growth in regions under electrical stimulation.

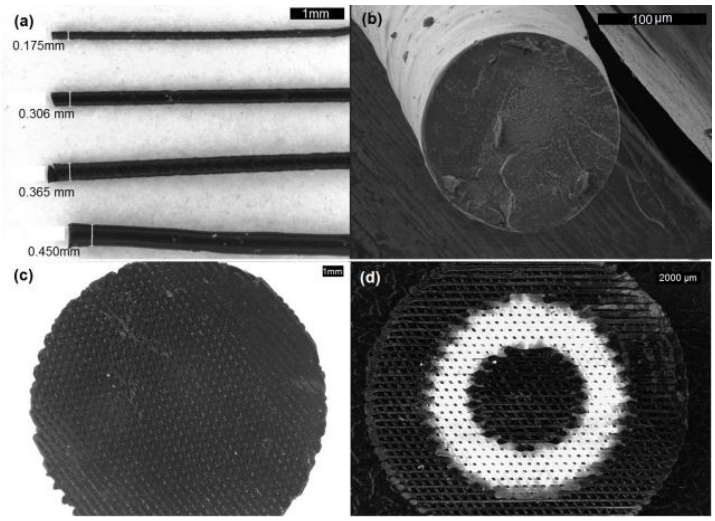

Fig.3. Printed graphene/PCl fibres with different diameters(a), Cross-section of extruded PCl fibres (b), graphene/PCl composite printed in the form of a scaffold (c) and graphene/PCl composite and pristine PCl printed in the form of combined scaffold (d).

3.1. Mechanical Properties

In general, the addition of graphene results in large increases in the tensile strength, yield strength and Young's modulus of the fibres. Graphene/PCl composite fibres exhibited an increase of up to 50% in tensile strength from 33.7 MPa to 50.6 MPa and a more than 160% improvement in tensile yield strength with less than 5% (w/w) graphene content. The Young's modulus of the fibres was also increased by more than 143% on addition of 5% (w/w) graphene. These results indicate a significant contribution of graphene to strength improvement, deformation resistance and stiffness of the fibres. Although the addition of graphene reduced the elongation at break of the composite, the extruded composites still exhibit reasonably high elongations at break of 1295%, indicating a flexible, strong fibre.

Table I. Mechanical properties of extruded fibres [300 – 365 µm] at different graphene contents.

Graphene Content [%]	**Tensile Strength [MPa]**	**Young's Modulus [MPa]**	**Elongation at Break [%]**
0	33.7 ± 0.7	2.3 ± 0.2	2251 ± 91
1%	48 ± 0.8	4.1 ± 0.3	1326 ± 76
2.5%	48 ± 0.8	4.7 ± 0.3	773 ± 54
5%	50.6 ± 0.9	5.6 ± 0.3	1295 ± 81

3.5. Cell Study

In order to test the cytotoxicity of the extruded fibres, electroactive PC12 cells were seeded onto the fibres. Fig. 4a shows differentiated PC12 cells exhibiting a neural morphology growing on the printed scaffolds. The green phalloidin stain shows that the actin cytoskeleton of the cells was typical of PC12 cells which have differentiated towards a neuronal phenotype. Fig. 4b shows cells adhered to adjacent fibres, with the bed of cells growing on the underlying microscope slide, demonstrating that the nerve cells preferentially adhered to and differentiated on the extruded fibres over the uncoated glass.

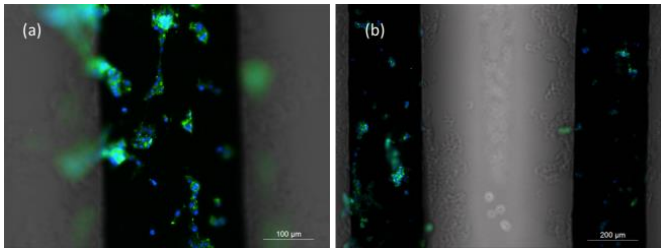

Fig. 4. Differentiated PC12 cells showing good adherence and morphology on a single fibre (a) and on two fibres and the glass substrate (b).

4. Conclusion

Covalently-linked PCl graphene composites are good candidates as materials for tissue engineering scaffolds due to their biocompatibility, biodegradability and physical properties. Here, the processability of the composites was investigated by plotting them in the form of fibres and scaffolds, as the addition of graphene to PCl did not affect the melting point or viscosity of the composites considerably. The extruded fibres show remarkable mechanical properties with more than 50% increase in tensile strength and up to 144% improvement in Young's modulus on the addition of less than 5% graphene filler. Biocompatibility was confirmed by the preferred proliferation of PC12 cells on the extruded fibres.

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