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Multilayer integration manufacture of graphene oxide based nerve scaffold

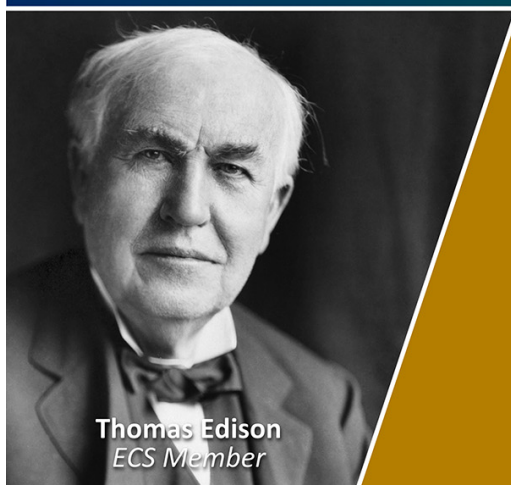
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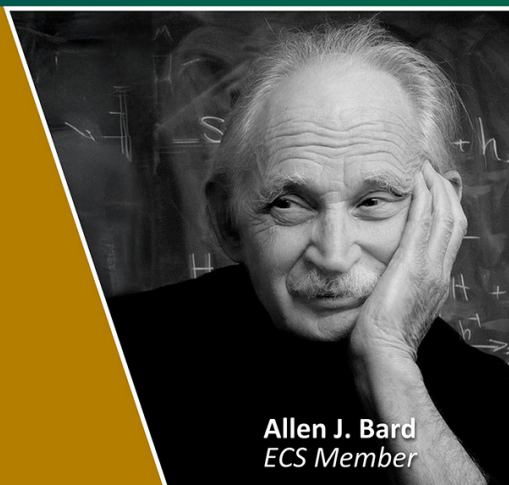
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Multilayer integration manufacture of graphene oxide based nerve scaffold

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Abstract. Graphene oxide is a kind of electrically conductive material with excellent biocompatibility for neural tissue engineering. The combination of laminin modified graphene oxide and polycaprolactone was evaluated in nerve scaffold fabrication. The integration manufacture style created multilayers and microporous structure in the scaffold. The multiple pore alignment facilitated free nutrient exchanges and also prevented alien tissue from penetrating into the lumen. The mechanical properties, including elongation at break and elastic modulus were improved in the presence of graphene oxide. The conductive scaffold also proved to provide a biocompatible environment for nerve cell viability.

1. Introduction

Peripheral nerves display certain regenerative abilities after mild and moderate insults. Nevertheless, they cannot be fully repaired in severe injuries, like large nerve defects without appropriate regeneration cues [1]. In clinical work, surgical bridging using autologous nerve transplantation is commonly used for considerable defects [2]. But this technique is restricted by three aspects, such as limited nerve resources, prolonged surgical time and potential damage to the donor sites. Therefore, it is of vital significance to look for synthetic scaffolds as ideal replacement. A nerve guiding scaffold is equipped with characteristics, including biocompatibility, structural stability and electrically conductivity [3]. Polycaprolactone (PCL) is a common biodegradable and structurally stable synthetic material used in tissue engineering. It is widely applied in neurons, cardiac muscles and bone tissue repair. As a basic substrate, it guarantees mechanical strength but it cannot induce any electrical conductivity [4].

Graphene oxide (GO) is an important electrically conductive material in the graphene family [5]. It is characterized by a single atom layer and amphiphilic functional groups. These epoxide, hydroxyl and carboxylic acid groups, along with the big aromatic structure enable GO to have a stable colloidal structure and closely interact with proteins, peptides and DNAs. Its excellent biocompatibility allows the relevant applications in different tissue regeneration [6-8]. GO has good electrical conductivity, which is significant to electroactive tissues. GO coating on the poly-lactide scaffold could greatly improve the proliferative and differential performance in neurite outgrowth [9]. The addition of 0.4% GO in the polyacrylamide mixed hydrogel enhanced Schwann cell viability and adhesion [10]. In this study, we intend to fabricate a GO based PCL scaffold modified by laminin and evaluate its surface and internal structure, mechanical properties as well as electrical conductivity. In addition, we investigate the potential compatibility of the scaffold for Schwann cells in the peripheral nerve tissue engineering.



2. Materials and methods

GO, PCL and laminin were obtained from Sigma Aldrich. GO nanoparticle was dissolved in PCL/dichloromethane and sonicated for 8 minutes. After that, we used an integration molding method for scaffold fabrication. 3D printer was used to add aligned micropores in the scaffold surface. We then characterized the scaffold surface morphology with scanning electron microscopy (SEM, Sirion 200/IAC, SJTU) and GO nanoparticle distribution with transmission electron microscopy (TEM, JEOL JEM-2010). In addition, we also evaluated the mechanical properties including elongation at break and elastic modulus using nanoindentation (Agilent, USA). We also measured the electrical conductivity with a Hall Effect Test System (Dexing Magnet Tech, China). After the scaffold characterization, we also performed cell counting kit 8 assay (CCK8, Beyotime, China) to evaluate the rat Schwann cell (RSC, cell bank of the Chinese Academy of Sciences, Shanghai, China) viability condition in laminin coated GO/PCL scaffold and PCL scaffold. All tests were repeated three times, and the results were shown as the mean \pm standard deviation. Unpaired student's t-tests were used for statistical analyses. A p value of 0.05 was significant.

3. Results and discussion

The fabrication process of laminin coated GO based PCL scaffold was illustrated in Figure 1. We prepared in advance a tubular mold, which was made up of concentric tubes. GO nanoparticle was evenly distributed in the surface of conduit. Then, we added aligned micropores using a three-dimensional printer. Finally, laminin was sprayed in the outermost surface of the scaffold. The multilayered design had many advantages. It could improve the space between different layers and thus add to the mechanical specialties. In addition, the microporous alignment in the surface facilitated scaffold biodegradation and assured the in vivo biocompatibility in a lengthy process. It also provided free exchanges of water, protein and other nutrients. Meanwhile, the appropriate porosity could prevent fibroblasts from entering the conduit and disturbing normal neurite extension.

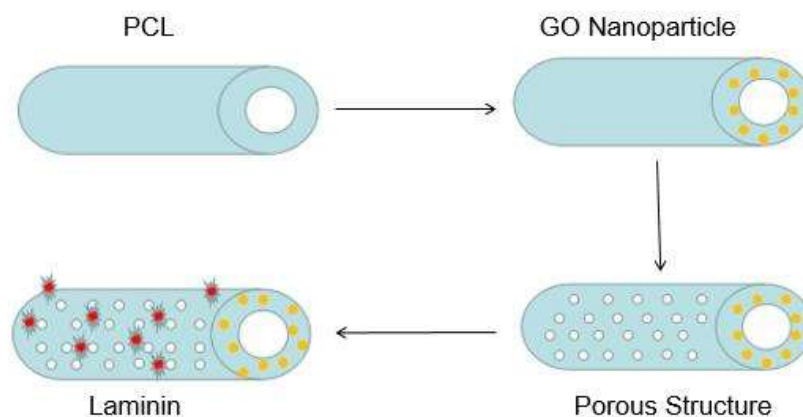


Figure 1. Integration molding manufacture of laminin coated GO/PCL scaffold.

We evaluated the distribution and structure of the GO nanoparticle in the laminin coated GO/PCL scaffold using TEM. GO nanoparticles were evenly distributed in the area shown at different magnification in Figure 2 and Figure 3. In addition, we characterized the surface morphology of the laminin coated GO/PCL scaffold using SEM. The relatively rough surface structure was shown in Figure 4. We also observed staggered layers due to the integration fabrication design.

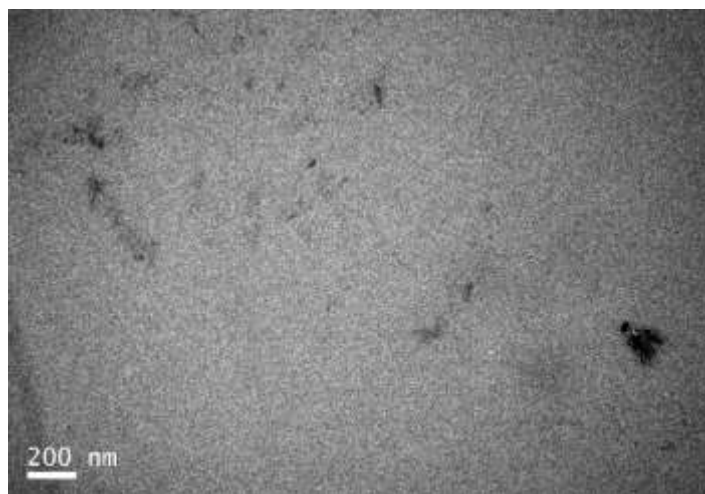


Figure 2. TEM images of GO nanoparticles (scale bar=200 nm).



Figure 3. TEM images of GO nanoparticles (scale bar=50 nm).

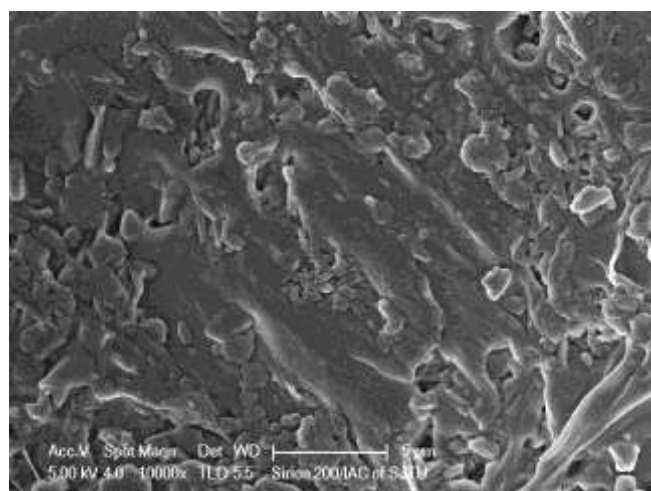


Figure 4. Rough surface of laminin coated GO/PCL scaffold.

We further assessed the mechanical characteristics of the laminin coated GO/PCL scaffold (Table 1). The mean elastic modulus of laminin coated GO/PCL scaffold (49.11 MPa) was significantly higher than PCL scaffold (32.06 MPa). It indicated that the addition of GO nanoparticle greatly enhanced the stiffness of the compound scaffold, which could ensure that the nerve scaffold did not collapse easily in the long-term in vivo environment. In addition, we used the elongation at break to revalidate the structural stability of the scaffold. The results showed that it was 44.2% for laminin coated GO/PCL scaffold versus 34.0% for PCL scaffold. Then, we evaluated the electrical conductivity of the scaffolds. PCL scaffold did not conduct electricity. The laminin coated GO/PCL scaffold showed excellent conductivity with the value of $4.56 \times 10^{-4} \text{ S cm}^{-1}$.

Table 1. Mechanical and conductive properties of laminin coated GO/PCL scaffold.

Scaffold	Conductivity (S cm^{-1})	Mechanical properties	
		Elongation at break (%)	Elastic modulus (MPa)
Laminin/GO/PCL	4.56×10^{-4}	44.2	49.11
PCL	/	34.0	32.06

After characterizing the properties of the scaffold, we performed CCK8 assay to evaluate the rat Schwann cell viability in different scaffolds. The schematic image of cell seeding on the scaffold was displayed in Figure 5. At different time points, including 24h, 72h, 120h and 168h, we found that there was no significant difference between laminin coated GO/PCL scaffold and PCL scaffold as was displayed in Figure 6. It showed that both scaffolds displayed excellent biocompatibility for cell proliferation.

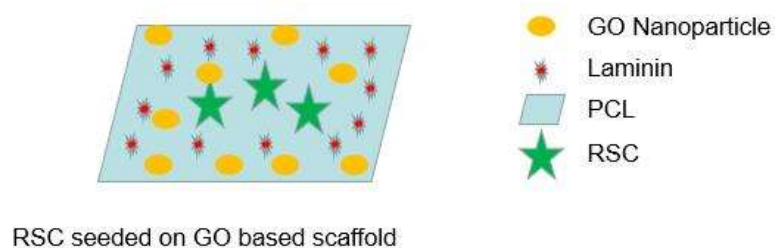


Figure 5. Schematic illustration of cell seeding on the scaffold.

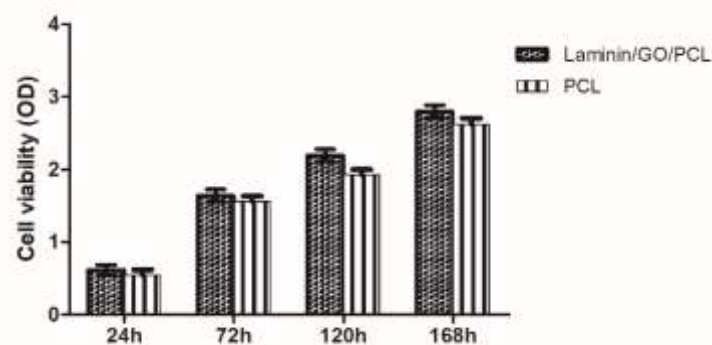


Figure 6. Cell viability evaluation using CCK8 assay.

4. Conclusions

In summary, the integration manufacture was used in the synthesis of the laminin coated GO/PCL scaffold. The technique significantly improved the mechanical properties of the scaffold by introducing the multilayer structure and microporous architecture, facilitating ideal nutrient exchanges and transport. More importantly, it guaranteed the structural stability and steady biodegradation for the scaffold, which was important to long-term nerve repair. The excellent electrical conductivity was confirmed by GO addition in the scaffold, which was beneficial to nerve regeneration. Furthermore, the biocompatible nerve scaffold offered ideal cell viability and was expected to play a bigger role in nerve tissue engineering.

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