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Carbon nanotubes increase the electrical conductivity of fibroblast-seeded collagen hydrogels

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Abstract

Carbon nanotubes are attractive as additives in fiber-reinforced composites due to their high aspect ratio, strength and electrical conductivity. In the present study, solubilized collagen Type I was polymerized in the presence of dispersed single-walled carbon nanotubes (SWNT) and human dermal fibroblast cells (HDF) to produce collagen–SWNT composite biomaterials with HDF embedded directly in the matrix. The resulting constructs, with SWNT loadings of 0 (control), 0.8, 2.0 and 4.0 wt.% SWNT, were cultured and electrical properties were evaluated in the frequency range 5–500 kHz at days 3 and 7. All collagen–SWNT hydrogel matrices underwent HDF-mediated gel compaction over time in culture, but the presence of SWNT significantly decreased the rate and extent of gel compaction. Viability of HDF in all constructs was consistently high and cell morphology was not affected by the presence of SWNT. However, cell number at day 7 in culture decreased with increasing SWNT loading. Electrical conductivity of the constructs varied from 3 to 7 mS cm^{-1}, depending on SWNT loading level. Conductivity increased uniformly with increasing wt.% of SWNT ($R = 0.78$) and showed a modest frequency dependence, suggesting that the electrical percolation threshold had not been reached in these materials. These data demonstrate that the electrical conductivity of cell-seeded collagen gels can be increased through the incorporation of carbon nanotubes. Protein–SWNT composite materials may have application as scaffolds for tissue engineering, as substrates to study electrical stimulation of cells, and as transducers or leads for biosensors.

Keywords: Composites; Single-walled nanotubes; Electrical conductivity; Collagen Type I; Fibroblasts

1. Introduction

Composite materials combine at least two separate phases to produce a new material with properties superior to those of the individual components. In the case of fiber-reinforced composite materials, a phase consisting of strong, stiff fibrous components is embedded in a more ductile matrix phase. The reinforcing phase can augment the mechanical properties of the composite material, but often this phase also exhibits higher thermal and electrical conductivity than the matrix phase, and as a result can increase the conductivity of the material as a whole. The main determinants of the degree to which a fiber additive can increase the electrical properties of a material are the intrinsic conductivity of the additive, the geometry of the additive and the loading level of the additive. Longer geometries and higher loading levels are more likely to produce longer individual conductive pathways in the material. As these pathways increase in length and become more interconnected, a point is reached at which electrical percolation occurs and the material typically exhibits a sharp increase in conductivity [23].

Carbon nanotubes are very attractive as additives in fiber-reinforced composites due to their high aspect ratio, conductivity and strength. In particular, individual single-walled carbon nanotubes (SWNT) typically have diameters of 0.7–2.0 nm but their lengths can be on the order of 1–10 μm, such that they have aspect ratios on the order of 1:1000 to 1:10,000 [1]. SWNT are essentially pure carbon

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polymers, with each carbon atom in the lattice bound covalently to only three neighboring carbon atoms. The highly symmetric beam-and-truss structure formed by the covalently bound carbon atoms gives high stability and strength, while retaining flexibility [11,35]. However, the fourth valence electron is shared and mobile, making nanotubes aromatic and allowing them to conduct electricity. Certain SWNT structures (i.e. allotropes) exhibit true metallic properties, with current-carrying capacities 1000 times higher than copper wires [9]. Nanotube loading levels of 1–5 wt.% in various synthetic polymer matrices have been shown to markedly improve electrical conductivity [8,20], however, a key to harnessing the full potential of carbon nanotubes in such applications is to align them to take advantage of their high aspect ratio. It is estimated that alignment of the nanotube phase could produce improved mechanical and electrical properties at loadings as low as 0.1 wt.% [7].

Materials that incorporate carbon nanotubes have been investigated for a variety of biomedical applications. For example, efforts to create novel nanotube-based assays, electrodes and sensors have accelerated in recent years [15,21,36]. A number of recent studies have focused on the development of composite materials incorporating carbon nanotubes to enhance the properties of synthetic polymers commonly used in biomedical applications [18,21,31]. A smaller number of studies have examined the possibility of creating nanotube-reinforced composites of naturally derived biopolymers such as silk fibroin [4] and chitosan [33]. Promising applications of such materials include their use as biomaterial scaffolds, sensors and delivery tools in the field of tissue engineering [12]. In particular, there are specific tissues in which electrical conductivity is an important functional feature, such as cardiac and neural tissue. It has also been suggested that electrical stimulation can cause physiological changes in a variety of cell types [6,14].

We have shown previously that carboxylated SWNT can be incorporated into cell-seeded hydrogel matrices consisting of the structural protein collagen Type I [16]. Collagen from tissues can be isolated and reconstituted into a fibrillar matrix under near-physiological conditions (pH, temperature, osmolarity), allowing living cells to be entrapped directly in the polymer matrix. The well-understood hierarchical self-assembly process that leads to collagen fibril formation also can entrap SWNT in the matrix as it polymerizes, resulting in the formation of collagen–SWNT composite materials. Our hypothesis is that entrapment of SWNT in collagen matrices will increase the electrical conductivity of these materials. The collagen provides a matrix that is stable and permissive of cell function, but that also can be reorganized by the living cellular component since cells can bind to the collagen that surrounds them and can actively remodel, augment or replace it. We have proposed that such protein–SWNT composite materials can be used in a variety of biomedical applications, such as scaffolds for tissue engineering and biosensors, and also may be useful as a way of directing the assembly of SWNT in three-dimensional structures for non-biological applications (e.g. circuit assembly). In the present paper we report on our recent work on creating and testing electrically conductive composites of reconstituted collagen Type I and carboxylated SWNT with embedded fibroblast cells.

2. Materials and methods

2.1. Cell culture

Human dermal fibroblast cells (HDF) were used in all experiments as a prototypical cell type, since this study was aimed at determining the feasibility of creating electrically conductive collagen hydrogels. HDF are well known to be able to remodel collagen gels, and therefore they are a good candidate cell type for creating the conductive materials we envision. In addition, HDF have relevance in certain soft tissue applications such as skin, tendons and ligaments. However, future experiments will also be conducted using tissue-specific cell types suited for particular applications. HDF were purchased (Cambrex Inc., Cambridge, MA), cultured according to the vendors specifications and harvested using trypsin (Cellgro Inc., Herndon, VA) between passage 4 and 10. Cells and collagen–SWNT constructs were cultured in Dulbecco's modified Eagle's medium (Cellgro) supplemented with 10% fetal bovine serum (HyClone, Logan UT), 2 mM L-glutamine (Cellgro), 100 U ml^{-1} penicillin (Cellgro), and 100 μg ml^{-1} streptomycin (Cellgro). The construct medium was changed every 3 days.

2.2. Construct preparation

SWNT produced via high-pressure carbon monoxide conversion synthesis (Carbon Nanotechnologies, Houston, TX) were functionalized with carboxyl groups by refluxing in a mixture of 5 M nitric acid and 5 M sulfuric acid for 2 h. This mixture was then diluted with water, filtered over a 0.2 micron filter and washed thoroughly with distilled water. This was followed by a wash with ammonium hydroxide and a subsequent water wash to obtain a neutral pH. The functionalized SWNT were then recovered from the filter and sonicated in pure water for 5 min to produce suspensions of carboxylated SWNT in water at concentrations up to 0.5 mg ml^{-1}.

The process for creating collagen–SWNT constructs is shown schematically in Fig. 1. HDF were combined with the appropriate concentration of cold SWNT solution, concentrated culture medium, 10% fetal bovine serum and acid-solubilized bovine collagen Type I (Sigma–Aldrich, St. Louis MO). This mixture was neutralized with 0.1 M sodium hydroxide and then 3.0 ml of the cold suspension was poured into each well of a standard six-well culture plate. Subsequent elevation of the temperature to 37 °C caused collagen gelation and formation of a cell-seeded, disk-shaped construct with an initial diame-