A conductive cell-imprinted substrate based on CNT-PDMS composite

Hanie Kavand¹, Mahdi Rahaie¹,*, Javad Koohsorkhi², Nooshin Haghghipour³, Shahin Bonakdar³

1. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, PO Box: 14399-57131, Tehran, Iran.

2. Advanced Micro and Nano Devices Lab, Faculty of New Sciences and Technologies, University of Tehran, PO Box: 14395-1561, Tehran, Iran.

3. National Cell Bank of Iran, Pasteur Institute of Iran, PO Box: 13169-43551, Tehran, Iran.

Running title: 3D cell-shaped electrodes for biomedical applications

* Correspondence to:
Tel.: +98 21 86093408; Fax: +98 21 88497324
mrahaie@ut.ac.ir
ABSTRACT

Cell function regulation is influenced by continuous biochemical and biophysical signal exchange within the body. Substrates with nano/micro-scaled topographies that mimic the physiological niche are widely applied for tissue engineering applications. As the cartilage niche is composed of several stimulating factors, a multifunctional substrate providing topographical features while having the capability of electrical stimulation is presented. Herein, we demonstrate a biocompatible and conductive chondrocyte cell-imprinted substrate using PDMS and carbon nanotubes (CNTs) as conductive fillers. Unlike the conventional silicon wafers or structural photoresist masters used for molding, cell surface topographical replication is challenging as biological cells showed extremely sensitive to chemical solvent residues during molding. The composite showed no significant difference compared to PDMS in regard to cytotoxicity while an enhanced cell adhesion was observed on the conductive composite’s surface. Integration of nanomaterials into the cell seeding scaffolds can make tissue regeneration process more efficient.

KEYWORDS: Bioengineering, Biomimetic; Elastomers; Nanotubes;
INTRODUCTION

As early as 1743, Hunter defined the unique structure of cartilage and noted that “cartilage disease is troublesome, cures more difficult than bone, and when destroyed, it is never recovered”.(1) The ineffective healing potential of cartilage is due to the absence of vascularization, lack of stromal cell population, and the poor mitotic activity of chondrocytes, which are the only residing cell type in cartilage.(2,3) To overcome the limitations existing in the in vivo tissue repair, several strategies have been proposed and practiced. For instance, nonsurgical treatments rely on pharmacological treatments such as anti-inflammatory medications or physical therapy, whereas surgical options include marrow stimulation (drilling, abrasion arthroplasty, and microfracture) and direct cells or tissue transplantation.(3) However, most efforts are now focused on the in vitro platforms where tissues are engineered in an in vitro system and later on, implanted at the defect site. Mesenchymal stromal cells (MSCs) are a promising cell source for tissue regeneration and have the potential to differentiate into multiple specialized cells including adipocytes, osteoblast, and chondrocytes.(4)

Cells in the cartilage niche are involved in cell-cell interactions, cell-ECM interactions, and are continuously affected by physical loadings. This highlights several critical considerations that need to be addressed for successful cartilage regeneration. Although a majority of chondrocytes exist as isolated cells in the cartilage ECM, it is noteworthy to mention that chondrogenesis is triggered by cell-cell interactions.(5) The second interaction level includes the cartilage ECM, which gives the tissue most of its mechanical characteristic, is divided into different zones, and mainly consists of water, collagen (mostly type II collagen), proteoglycans, non-collagenous proteins, and glycoproteins.(3) Finally, physical loadings are considered as the third influential parameter involved in cartilage regulation. Role of cell deformations, compression, hydrostatic pressure, and electromechanical inductions on cartilage metabolism have been extensively investigated.(6–9) Although cartilage is known to be mainly driven by mechanical loadings, the electric potentials generated during mechanical to electrical transduction are of considerable importance as well.(10) Moreover, the pericellular matrix (PCM) in chondrocytes has a higher proteoglycan content, thus during loadings and tissue deformations, the physiochemical and osmotic environments of the
chondrocytes are affected and electrokinetic phenomena including electric streaming potentials arise.(11,12) The successful studies and the necessity of physical cues for normal tissue function have strengthened the theory and the applicability of physical stimuli for tissue regeneration.

Much attention is drawn to the design and selection of biomaterials for cartilage tissue engineering as the prominent role of cartilage ECM in maintaining and promoting the functionality of chondrocytes is becoming increasingly apparent. The idea of imprinting molecular features in polymers emerged as these structures found enormous applicability in separation, recognition (mimicking antibody and receptor binding sites), and bio-sensing.(13) Bacteria and eukaryote cell imprinting further progressed the initial imprinting concept.(14,15) Jeon and colleagues used alternating current (AC) electric field to transfer MG63 cell patterns to PDMS.(15) The electric field creates motion to the PDMS elastomer and ensures efficient pattern transfer. They observed a significant cellular activity upon the re-culture of MG63 cells on these substrates. Cell-imprinted substrates were further used for applications such as cell differentiation studies.(16–18) Overall, engineered micro-and nano-scaled scaffolds have been of particular interest for tissue engineering applications as these topographies are believed to control complex cell functions (see (19–21) for review).

Since the discovery of CNTs in 1991, considerable progress had been made in the field of electronics and medicine.(22) Owing to their unique electrical and mechanical properties and their high aspect ratio, CNTs are incorporated in several applications such as transistors, supercapacitors, biosensors, drug delivery carriers, photothermal and photodynamic therapy, imaging, and tissue engineering.(23,24) CNT-poly(dimethyldiallylammonium) multilayered nanocomposites was reported to provide regulatory signals for neural differentiation and electrophysiological maturation.(25) Electric currents were used to induce osteogenesis in MSCs seeded on silicon-based composite containing collagen and CNT fibers.(25) Besides the electrical advantages, CNTs can have structural roles as well. An example of this role can be seen in Ren and colleagues work on cardiac tissue.(13) Their results show that upon the application of super-aligned CNTs sheets, cardiomyocytes morphology was elongated, aligned, and the synchronous cell contraction was achieved.
Incorporation of CNT into polydimethylsiloxane (PDMS) elastomer provides a conductive, stretchable, and patternable membrane. CNT-PDMS composite had been successfully applied for nanogenerators, optical technologies, electroencephalogram (EEG) electrodes, and electromechanical cell stimulation.(26–29) However and to our knowledge, no report exists on the fabrication of a conductive cell-imprinted substrate. Herein, we aim to fabricate a conductive cell-imprinted topography for cell-based studies. We try to take advantage of the molding capability of PDMS polymer and the conductivity of CNTs to fabricate substrates that could have the potential for chondrogenic differentiation studies.

Biological cells are extremely sensitive to solvents that are frequently used for CNTs dispersion. This results in decreased fidelity in replicating cell-surface topographical cues if the solvent remains in the composite mixture prior to molding/curing. Upon fabricating the conductive cell-imprinted surface, results showed no significant difference between the PDMS and the CNT-PDMS substrates in terms of cytotoxicity. Moreover, cell adhesion was enhanced on CNT-PDMS (flat) substrates. As the integration of CNTs affects PDMS’ innate properties, the composite’s characteristics should be carefully considered for future study designs.

**EXPERIMENTAL**

**Materials**

Phosphate-buffered saline (PBS), DMEM/F12, fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Gibco, Life Technologies. Collagenase D was purchased from Roche. Trypsin-EDTA solution (0.25%), (3-Aminopropyl)triethoxysilane (APTES), glutaraldehyde, chlorotrimethylsilane (TMCS), 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich. n-Hexane and isopropanol were obtained from Merck (Germany). All reagents were of chemical grade and were used as received.
**Chondrocyte isolation and seeding**

Chondrocytes were isolated from the cartilage of a rabbit obtained from a slaughterhouse (for this type of study formal consent is not required) as previously described.(30) Briefly, tissue was minced, washed (PBS + penicillin/streptomycin), and kept in trypsin-EDTA solution for 1 h. Trypsin-EDTA solution was then replaced with media containing DMEM/F12 and collagenase D (0.5 mg.ml⁻¹) and incubated overnight with gentle agitation in an incubator (37°C, 5% CO₂). After cell liberation from the tissue ECM, cells were washed with complete media (DMEM/F12 + 10% FBS), and seeded on glass slides. To overcome the hydrophobicity of the glass surface and to facilitate cell attachment, glass slides were activated by O₂ plasma (0.6 mbar, 30 sec, 100 W) and functionalized with APTES prior to cell seeding.

**AD-MSCs isolation and culture**

Adipose-derived mesenchymal stromal cells (AD-MSCs) were isolated from the white subcutaneous adipose tissue (WAT) of the same rabbit as previously described.(31) Briefly, tissue was washed three times with PBS, minced, and digested in collagenase solution (0.5 mg.ml⁻¹) (50 min, 37°C, 5% CO₂). None digested tissue was collected and the stromal vascular fraction (SVF) was separated by centrifugation. The pellet was washed before suspension in complete media and seeding.

**Fabrication of CNT-PDMS cell-imprints**

To prepare cell templates for imprinting, chondrocytes were fixed with 4% glutaraldehyde after 24 h of seeding. PDMS (Sylgard 184, Dow Corning) cell-imprinted substrate was prepared by mixing the base and the curing agent at a 10:1 weight ratio. To prepare conductive cell-imprinted substrates, pristine MWCNTs (multi-walled carbon nanotubes, outside diameter: 20-30 nm, length: 10-30 μm, electrical conductivity: >100 s/cm, US Research Nanomaterials, Inc.) were utilized as the conductive filler. CNTs were dispersed in n-Hexane to make CNT-PDMS composites with varying filler concentrations (1, 1.5, 2, 3, and 5 wt.%). CNTs were sonicated in n-Hexane (on ice) for 1 h to achieve uniform dispersion. After adding the PDMS base polymer to the dispersed CNTs, the mixture was
initially sonicated (2 h) and then magnetically stirred for 3 h on a hotplate at 50°C to ensure a homogenous dispersion of CNTs in the PDMS base polymer before adding the curing agent. Fixed cells were then molded by the CNT-PDMS matrix and cured at 60°C for 6 h. As PDMS is known to absorb hydrophobic molecules(32), the CNT-PDMS cell-imprints were kept in DI water for 3 days (water changed twice a day) to ensure that no residues of solvent remains in the substrate that would affect cell viability. As this study aims at the fabrication of conductive cell-imprints, analyzing the cell behavioral effects induced by cell-imprinted topographies is not discussed. Cell toxicity and cell adhesion analysis were conducted on PDMS and CNT-PDMS substrates molded from polished silicon wafers. To ease PDMS stripping, silicon wafers were treated with TMCS.

**Imaging**

Chondrocytes were inspected with an optical surface profiler/interferometer (Veeco Wyko NT1100). Cell-imprinted topographies were visualized with atomic force microscope (AFM; DME DS 95 Navigator 220), scanning electron microscope (SEM; HITACHI S-4160 and Zeiss LEO 1550), and optical microscope (Nikon Optiphot 200). All samples, except the CNT-PDMS cell imprints, were sputtered with gold before SEM imaging. CNT-PDMS cell-imprint images were acquired without any gold coatings and imaged at low acceleration voltage (0.8 kV).

**Electrical characterization**

A four-point probe measurement technique was applied to measure the current-voltage (I-V) curves of the prepared PDMS composites (Keithley 2602A SourceMeter).

**Morphological analyses**

For cellular morphology evaluations, MSCs were cultured on tissue culture plate (TCP, Greiner), flat PDMS, and CNT-PDMS substrates. Cells were inspected 24 h after cell seeding (cells were fixed with 4% glutaraldehyde).
Cell viability

The viability of cells was determined using the MTT assay. Cells were seeded at a $2 \times 10^4$ density on TCP, autoclave sterilized PDMS and CNT-PDMS substrates. Cells were incubated (37°C, 5% CO$_2$) for 24 h to allow cell attachments. Cells were then washed with PBS, MTT solution was added, and cells were stored in the incubator for 4 h. After the formation of formazan crystal, cells were washed with PBS, and finally, the crystals were dissolved in isopropanol. The absorbance was measured at 545 nm using a spectrophotometer. A one-way ANOVA test was performed (GraphPad Prism 7 software) to ascertain statistical significance between the expression levels. P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Cell-imprinted substrates

Bioengineered scaffolds are designed to mimic the physiological factors present in tissues and promote efficient tissue regeneration. We developed a CNT-PDMS cell-imprinted substrate with enhanced electrical properties. Cell imprinting is regarded as a general technique and the CNT-PDMS composites can be used to replicate any cell type into an elastic and conductive polymer. Figure 1a,b shows the rabbit chondrocytes were are used as the cell model for CNT-PDMS cell-imprint fabrication.

During the imprinting process, the polymer is cross-linked at the interface of the cell and the polymer while the cell surface acts as the template for molding. After the subsequent polymer detachment, topographical features are transferred to the polymer, which mimics the topographical features present on the template cell. This platform provides nanometric physical recognition sites that may induce certain cellular activities upon consecutive cell culture. PDMS is an excellent imprinting polymer and

This article is protected by copyright. All rights reserved.
allows a high-resolution replication of the cell topography. Figure 1c shows an AFM image of PDMS chondrocyte cell-imprint. As can be seen, the 3D cavity structured on the PDMS imitates the cellular topography. During initial cell seeding (creating the master), cells sometimes adhere to one another and result in a complex topography on the PDMS substrate (Figure 2a). Nevertheless, the exposed physical features of individual cells will be transferred to the PDMS. SEM image of individual cells replicated into the PDMS substrate is shown in Figure 2b.

**CNT-PDMS cell-imprinted substrates**

To fabricate conductive cell-imprint substrates, the conventional PDMS imprinting process is modified. This modification is largely because CNTs are prone to agglomeration. The electrical implementation of nanocomposites relies upon the dispersion of nanomaterial through the polymer matrix. Although the large surface area of CNTs can be a desirable factor for surface modification purposes, it also produces a strong attractive force between the tubes that result in unwanted agglomeration (Figure 3a). To overcome this clustering and enhance the uniform dispersion of CNTs into the PDMS before polymerization, CNTs should be very well dispersed into the base polymer. The CNTs tend to agglomerate due to the Van der Waals force; hence to prevent CNT agglomeration in the CNT-polymer composites, several functionalization methods have been presented.(33) However, Oh and colleagues discuss that functionalized MWCNTs have lower conductivity compared to pristine MWCNTs.(34) According to them, the lowered conductivity is due to the degradation of MWCNTs subsequent to covalent functionalization and that most functionalizing materials are insulators and the contact resistance between conductive fillers is large.

The CNT-PDMS composites used for molding applications are usually casted before solvent evaporation and the solvent is gradually evaporated during the curing process. Regardless of the damaging effects of the solvent on the cell structure (Figure 3b) in the cell-imprinting process, we also observed an altered electrical property along the PDMS thickness (data not shown) that could be due to the non-simultaneous solvent evaporation. To overcome these problems, the CNT-PDMS composite was prepared and enough time was given to the solvent to evaporate before cell molding.
using a manual film applicator. Figure 4a shows well-dispersed CNT fillers in a PDMS chondrocyte cell-imprinted substrate. The smoothness of the composite’s surface is not different from the PDMS surface (Figure 4b). This shows that the CNTs have fully incorporated into the PDMS matrix. Other studies also report a smooth surface with no defect or void in the CNT-PDMS surface. (35) In our study, however, the presence of artifacts on the master’s surface (residuals from cell seeding and fixation procedure) sometimes creates an uncontrollable roughness on the replicated substrates that can be ignored.

**Electrical properties**

One of the reasons that make CNTs an interesting candidate for developing conductive composites, is their high aspect ratio and surface area. This characteristic helps in achieving electrical percolation at lower concentrations in composites. The electrical percolation threshold is defined as the critical concentration of a filler that shows an extreme increase in the electrical conductivity of the polymer. (36) Different CNTs (such as shape and size) and procedures result in a varied percolation threshold seen in publications. We prepared CNT-PDMS composites with filler concentrations of 1, 1.5, 2, 3, and 5 wt.%. The current-voltage (I-V curve) characteristic of PDMS with different CNT filler concentration was studied (Figure 5). The graph shows an eventual nonlinear dependence of electrical conductivity to CNT concentration, suggesting that the conduction mechanism does not obey Ohm’s law. The percolation threshold is seen at 2 wt.% filler content. The mechanism suggested for the electrical behaviors at this concentration is suggested to be the inter-particle tunneling and the formation of percolation networks. (37) The CNT-PDMS composite at 3 wt.% filler concentration was selected for further analyses (Figure 4). The uniform dispersion of CNTs ensures a good conductivity while the replicated topography can act as a cell-seeding platform for further cell-based studies.

While taking advantage of the electrical conductivity of CNTs in preparing conductive polymers, it is important to consider the alterations in mechanical properties of CNT incorporated composites. A homogenous dispersion of CNTs in the composite’s matrix can yield higher conductance while keeping the mechanical characteristic of the substrate minimally affected. CNTs have a unique
strength of approximately 130 GPa, which is attributed to the intrinsic strength of carbon networks. (38) Depending on the filler concentration, the composite can have various mechanical properties. Xue and colleagues showed that the addition of nanotubes stiffens the PDMS composite and Young’s modulus of the membrane increases as the filler concentration is increased up to 5% and then the modulus decreases as the concentration reach up to 10% but still remains higher than the PDMS membrane. (35) Accordingly, CNT-PDMS cell-imprints are different in both electrical and mechanical properties compared to the PDMS cell-imprints. As these properties cannot be controlled separately, analyzing the effect of the substrate’s topography and electrical stimulations interfere with the biomechanical pathways triggered by the substrate’s stiffness. Cells sense and respond to a substrate’s stiffness (39); therefore such substrates would be more efficient for studies where a rigid substrate is needed. Cartilage shows regional variation in stiffness and mechanically adapts itself; regions with high-stress levels become stiffer while the areas with lower stress are softer. (40) This varied ECM structure and stiffness help in maintaining cellular organization in tissue, for instance, a higher stiffness in the interterritorial matrix compared to the territorial matrix-pericellular matrix, force cells to organize in columnar structures upon division. (41) Chondrocytes reside in an enclosed region called the pericellular matrix (PCM), which is biochemically defined as a type VI collagen-rich space that also contains high concentrations of proteoglycans, fibronectin, and types II and IX collagen. (42) The PCM’s elastic modulus had been reported to range between 13-75 kPa in porcine and 27-205 kPa in human cartilage. (12) Collagen is an abundant protein in the ECM and has high tensile stiffness. Wenger and colleagues measured Young’s modulus of individual type I collagen fibrils and found that the modulus was in the range of 5 GPa to 11.5 GPa. (43) They explained the observed variation in the values by the difference in individual fibrils, the dehydration state, and the uncertainty in the measurements. The question that remains to be answered is whether a cell experiences the local or the global stiffness of the tissue. It should also be noted that the PDMS membrane’s (mixed at 10:1 ratio of the base and the curing agent) Young’s modulus cured at temperatures from 25°C to 200°C is reported to be between 1.32 MPa to 2.97 MPa, respectively. (44) Another study reports an elastic modulus of 2.61 MPa for PDMS sheets (10:1, 2.5-3 mm thick) baked
at 65°C for 1 h. Regardless of the curing temperature or the base/curing agent ratio, which directly affect the mechanical properties of PDMS, these results indicate that the PDMS cell-imprints are already above the reported stiffness level of cartilage. Despite this difference, PDMS cell-imprints have been successfully used to differentiated MSCs into the chondrogenic lineage and morphological response of chondrocytes on negative and positive chondrocyte bio-imprints had been studied.

**Cell adhesion**

PDMS is a biocompatible polymer, however, because of its hydrophobic nature it impedes efficient cell attachments and spreading. When MSCs were cultured on a non-plasma or protein treated PDMS surface molded from a Si wafer, they exhibited a less flattened morphology (Figure 6a). These cells formed aggregates (Figure 6a-i) and did not attain their 2D cultured phenotype, which is a spindle-like morphology. Furthermore, cellular protrusions in these cells are not well separated and filopodia are not defined (Figure 6a-ii). On the other hand, MSCs’ cellular morphology on the CNT-PDMS substrate was more spread (Figure 3-b-i,ii) with definable cell membrane protrusion (Figure 6b-iii). This morphology is similar to the MSCs’ morphology cultured on TCP (Figure 6-c).

This result is interesting as it indicates that cell spreading is conducted by another possible mechanism, which is possibly not related to the chemical state of the substrate (for example the hydrophobicity). As indicated earlier, PDMS is a hydrophobic polymer. CNTs are hydrophobic nanomaterials and the incorporation of PDMS and CNTs creates a hydrophobic composite as well. It has been shown that by increasing the CNT concentration in a CNT-PDMS composite, the water contact angle increases and thus an enhanced hydrophobic surface is achieved. In fact, the reason for the more spread morphology on the CNT-PDMS substrate could be due to the substrate’s stiffness. Evans and colleagues results suggest that cell attachment on PDMS is independent of the substrate’s stiffness and only the cell spreading and proliferation is affected by the substrate’s stiffness. In an experiment conducted on the cellular behavior of SF295 cell line on ZnO films, Choi and colleagues showed that difference in the substrate’s conductivity affects cellular morphology. They suggest that highly conductive surfaces facilitate cell adhesion to the substrate through improved electrostatic interactions. These results suggest that conductive substrates can...
improve cell adhesion on PDMS substrates without any surface modifications processes such as plasma treating or protein coatings. We may postulate that the improved attachment on the CNT-PDMS cell-imprint is partly due to the enhanced electrostatic interactions.

**Cytotoxicity**

Carbon-based nanomaterials are generally regarded safe and this has extended their applications in biomedical studies. However, there are toxicity concerns about the direct contact of cells with MWCNTs when the nanomaterials are uncapped, needle-like, short, and dispersed in a solution. The CNTs’ chemistry (pristine and functionalized), length, and the dispersing agents also influence apoptosis and necrotic cell death. Cytotoxicity reports mainly focus on the dispersed state of the CNTs in solutions or when cells are in direct contact with CNTs. However, in a CNT-PDMS composite, CNTs remain trapped in the polymerized matrix. This inhibits cells to be in direct contact with CNTs. Moreover, the rheological properties of PDMS that give its unique molding capability, prevent the direct contact of the cell membrane with the CNTs as the nanostructures are coated with PDMS and their Brownian motions are confined within the matrix.

Our results from the cell viability analysis (Figure 6d) show a significant difference between cells cultured on a typical TCP and cells seeded on PDMS ($p \leq 0.05$) and CNT-PDMS cell-imprints ($p \leq 0.01$). As MSCs adhere perfectly to TCP, cell viability results were compared to it. After 24 h of culture, the cell viability on PDMS substrates and CNT-PDMS substrates (3 wt.%) were 63.05±7% and 69.34±12%, respectively. Although cell spreading was enhanced on CNT-PDMS surfaces, no significant difference was observed on the cell viability values of PDMS and CNT-PDMS substrates. It had also been reported that CNT-PDMS composites with filler concentrations less than 6 wt% have a biocompatibility rate equivalent to that of pure PDMS.
CONCLUSION

In this study, a conductive cell-imprinted scaffold composed of CNTs and PDMS was fabricated from chondrocytes as a cell-template model. Electrical percolation was observed at 2 wt.%. Incorporation of CNTs into the PDMS elastomer enhanced cell spreading compared to the PDMS substrate. As the CNT-PDMS composites vary in mechanical characteristics compared to the PDMS substrates, it is of importance to choose a relevant cell-study paradigm for analyzing cellular behaviors induced by cell-imprinted topographies. These biomimetic substrates can be an interesting platform for mechano-electrical stimulation of cells for tissue engineering applications.

Conflict of Interest:

The authors declare that they have no conflict of interest.
REFERENCES


This article is protected by copyright. All rights reserved.


of the American College of Rheumatology 2009, 60, 771.


Figures

**Figure 1.** Chondrocyte cell-imprints. (a) Optical image of chondrocytes fixed after 24 h of seeding. (b i,ii) Light interferometry micrographs showing the surface topography of chondrocytes. (c) AFM contact-mode image (2D) of PDMS chondrocyte imprint, a single cell’s imprinted topography rendered in 3D, and the topography graph from a cross-sectional analysis.
**Figure 2.** SEM micrographs of PDMS chondrocyte cell-imprints surface. (a) Imprinted surface from a dense populated area, and (b) single cell transferred patterns.

**Figure 3.** SEM micrographs showing (a) agglomerated CNTs in the PDMS chondrocyte cell-imprint with a perfectly cell replicated cavity. (b) In the presence of the residual dispersing solvent, cell surface is damaged and surface topographies are not transferred with fidelity.

**Figure 4.** CNT-PDMS composite cell-imprints (at 3 wt.%). (a) SEM micrographs showing the surface and the embedded CNT fillers. (b) AFM image showing cell-imprinted cell morphology on the CNT-PDMS substrate.
Figure 5. Current-voltage characteristic curves of CNT-PDMS composite for different percentiles of CNT fillers (0, 1, 1.5, 2, 3, and 5-wt.\%).

Figure 6. Morphology of MSCs cultured on (a) PDMS, (b) CNT-PDMS (3 wt.\%) substrates, and (c) tissue culture plate (TCP) surface. Arrow points to cell membrane protrusions, the filopodia. (d) Cell viability results after 24 h culture. * $p \leq 0.05$, ** $p \leq 0.01$, ns indicates not significant, One-Way ANOVA.