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Biocompatible and biodegradable fluorescent microfibers physiologically secreted by live cells upon spontaneous uptake of thiophene fluorophore

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Live cells are able to form multifunctional and environmentally responsive multiscale assemblies of living and non-living components. We have recently described results concerning a unique approach to introduce supplementary properties, fluorescence in particular, into fibrillar proteins produced by live fibroblasts and extruded into the ECM. In this paper we demonstrate that the physiological secretion of fluorescent nanostructured microfibers upon spontaneous uptake of the appropriate fluorophore, extends to living cells derived by different tissue contexts. We also show that live cells seeded on the fluorescent microfibers undergo a different fate in terms of cellular morphology, cytoskeleton rearrangement and viability. These results suggest that the microfibers, which are biocompatible and biodegradable, could be used as multiscale biomaterials to direct cell behaviour.

Introduction

A living cell contains complex substructures that are spatially and functionally self-organizing[1] and present unique characteristics useful for materials production and patterning. Our understanding of these systems and their dynamics is largely dependent on the possibility to visualize their presence inside the cells, in particular by means of fluorescence imaging. To this aim a variety of organic and inorganic tools have been developed spanning from fluorescent proteins[2] to nanoparticles,[3,4] quantum dots[5] and small organic molecules.[6] A great advantage of the latter is the possibility to employ the methodologies of synthetic chemistry to tailor their optical properties and to direct the position of the fluorophore inside live cells by means of appropriate functionalization.[7]

An emerging class of probes for biochemical imaging is that of thiophene oligomers and polymers. K. P. R. Nillson *et al.* have reported that a charged quinquethiophene can mark amyloid fibers[8] *in vitro* and *in vivo* and it is also able to discriminate between various types of cells.[9] Recently, we have reported that some cell-permeant and biocompatible thiophene fluorophores have the capability to spontaneously cross the cell membrane without the need of any type of vector and selectively recognize specific proteins inside living cells.[10-12] In particular, the green fluorescent thiophene fluorophore DTTO - namely 3,5-dimethyl-2,3'-bis(phenyl) dithieno[3,2-b;2',3'-d]thiophene-4,4-dioxide (see **Figure 1**) - is capable to spontaneously cross the membrane of live embryonic fibroblasts (3T3) and be specifically recognized by the hydroxyproline component of protocollagen polypeptide chains and be incorporated via nonbonding interactions during the self-assembly of protocollagen into triple helices. The process leads to the secretion of helical fluorescent microfibres subsequently extruded into the extracellular matrix.[11] DTTO being a semiconducting molecule, the collagen-DTTO microfibers are also electroactive.[12]

Here, we report that the capability of DTTO to spontaneously cross the membrane of live cells, be recognized by specific proteins and physiologically form fluorescent protein(s)-DTTO microfibers extends to mouse neuroblastoma cells (B104). The interest in testing the B104 cells line is in the fact that these cells do not produce collagen, thus the protein(s) targeted by DTTO must be of a different nature. We show that QTOF analysis of the fluorescent microfibers isolated from the cell context indicates that the fluorescent microfibers are mainly made of vimentin,[13] a fibrillar protein that is overexpressed in various types of cancers[14] as well as in the Alzheimer's disease[15] and that it is likely to play an important role in cancer cells invasion.[16] We also report that in view of their possible use as innovative biomaterials, we have tested the fluorescent collagen-DTTO microfibers produced by fibroblasts and vimentin-DTTO microfibers produced by neuroblastoma cells as substrates on which live 3T3 fibroblasts, C2Cl2 myoblasts and B104 neuroblastoma cells were seeded. We show that the protein-DTTO microfibers have dramatically different effects on the cells in terms of cellular morphology, cytoskeleton rearrangement and viability. These effects span from cells death to microfibers fragmentation into pieces by the cells and subsequent internalization without any adverse effect on cells viability. These results suggest that the physiologically produced protein(s)-DTTO microfibers could be employed to direct cell behaviour and, in prospect, also be active bioagents delivery systems and be used in tissue engineering applications.

Materials and Methods

DTTO was synthesized and characterized according to already described modalities.[11]

Cell culture. Mouse neuroblastoma cell cultures (B104) and mouse embryonic fibroblasts (3T3) were maintained in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 mg/mL streptomycin, 5% L-glutamine and 5% sodium pyruvate. Mouse myoblast cells (C2C12) were maintained in growth medium: DMEM medium supplemented with 15% FBS, 50 U/mL penicillin, 0.05 mg/mL streptomycin, 4 mM glutamine and 10 mM HEPES solution. Cells were grown in in a humidified incubator at 37°C, 5% CO_2, and 95% relative humidity. Upon reaching confluence, cells were passaged using 0.25% trypsin-EDTA. Cells at passages 3-10 were used in the following experimental assays.

Live Neuroblastoma cell labelling and LSCM monitoring.

Mouse Neuroblastoma cells (B104) were seeded at a density of 100,000 cells in tissue culture plate in 1 mL of complete culture medium. DTTO dye was dissolved in the minimum amount of DMSO in order to obtain a stock solution and then was administered to cells by adding the appropriate dilution in DMEM serum free to obtain the final concentration of 0.05 mg/mL and incubated at 37°C in 5% CO_2, 95% relative humidity for 1h. At the end of incubation period unbound dye was removed washing the cell cultures with DMEM medium serum free. The samples were examined after 1, 24, 48, 72 hours and 7 days upon treatment with DTTO by laser scanning confocal microscopy (LSCM). Confocal micrographs were taken with Leica confocal scanning system mounted into a Leica TCS SP5 (Leica Microsystem GmbH, Mannheim, Germany), equipped with a 63X oil immersion objective and spatial resolution of approximately 200 nm in x-y and 100 nm in z.

Isolation of fluorescent microfibers from cell lysates.

Fluorescent microfibers inside the cells were purified by whole cell lysates in 50 mM Tris HCl, pH 7.4; 1% Triton X-100; 5 mM EDTA; 150 mM NaCl, 1 mM Na_3VO_4; 1 mM NaF; 1 mM phenylmethylsulfonyl fluoride (PMSF), in the presence of protease inhibitor cocktail (10 µM benzamidine-HCl and 10 µg each of aprotinin, leupeptin and pepstatin A per ml) followed by incubation at 4°C. DTTO-conjugates were left to decant, harvested into fresh reaction tubes, washed three times with fresh lysis buffer by centrifugation, and stored at 4°C or -80°C until performing AFM investigations.

SDS-PAGE.

Samples dilution of DTTO-microfibers conjugates into SDS-loading buffer (1:1) were separated on SDS-polyacrylamide gels without prior heating. Resolved protein bands were visualized by Coomassie staining (Sigma Chemical Co., St. Louis, MO, USA), according to manufacturer's instructions.

Tryptic digestion and Mass spectrometry analysis.

Fiber pellets isolated by mouse neuroblastoma cells (B104) were resuspended with Urea 8 M and treated with 5 µl of ammonium bicarbonate (AMBIC) 100 mM, reduced with Dithiothreitol (DTT 10 mM, 1µl in AMBIC 100 mM) at 56°C for 30min and alkylated with iodoacetamide (55 mM, 1 µl in AMBIC 100 mM) at room temperature in the dark for 1 h. The resulting protein mixture was digested with TPCK-modified sequencing grade trypsin (final ratio of enzyme to substrate 1:50 w/w) at 37°C overnight. Samples were then acidified with 1 µl 5% formic acid (FA) solution and dried in a vacuum evaporator. Trypsinized microfibers were resuspended in 30 µl of 1% FA/acetonitrile 98:2 solution. Chromatographic separation was performed on a high capacity loading chip, with a 150 mm, 300Å, C18 column prior to a desalting step through a 160 nL trap column; injected sample (2 µl, 0.8 µg) was loaded on the trap column with a 4 µl/min 0.1% FA:ACN 98:2 phase; after 3min, the pre-column was switched in-line with the nano flow (400 nl/min, phase A: water:ACN:FA 97:3:0.1, phase B: ACN:water:FA 97:3:0,1), equilibrated in 3% B. The peptides were eluted from the RP column through the following gradient: 3-45% B over a period of 35 minutes, 45-90% in 7 min, 90% B hold for 5 min, and back to 3% B in 3 min - a total of 60 min of runtime, including a 10 min post-run reconditioning step.
Mass spectrometry analyses were performed by ESI-Q-TOF Accurate-Mass G6520AA (Agilent Technologies), controlled by MassHunter (v. B.02.00) and interfaced by a CHIP-cube to an Agilent 1200 nano-pump. Centroided MS scan spectra were acquired in positive mode in the range of 300 to 1700 Da with a 6 Hz sampling rate; top 5 ions, preferring +2 and +3 species, were selected for MS/MS analysis, setting an active exclusion of the same precursor after 1 spectra over 0.15 minutes. Tandem mass spectra (N2 CID cell) were recorded in the mass range 50 to 1700 Da with a sampling rate of 3 Hz. Collision energy was calculated according to the following expression: [3.6 (m/z / 100) - 3] V. Automatic QToF calibration was performed before each run. MassHunter (B02.00, from Agilent Technologies) produced mzData.xml raw data that were searched against SwissProt (v. 57.15) database using an in-house Mascot Server (v. 2.3, Matrix Science, UK) with the following settings: 30 ppm parent ion tolerance, 0.15 Da fragment ion tolerance, semitryptic cleavage with one allowed missed cleavage, carbamidomethyl-cysteine as fixed modification and oxidised methionine, lysine and N-terminus carbamylation as variable modifications, narrowing the search to the rodent proteome. A forward-decoy concatenated protein sequence database was used to evaluate the search false discovery rate.

Immuno co-localization of vimentin.

The fluorescent microfibers isolated by mouse neuroblastoma cells (B104) were incubated with anti-vimentin monoclonal antibody (1:150, Sigma-Aldrich, USA) in PBS buffer at 37°C for 1 hour. Primary antibody was revealed using TRITC conjugated anti-mouse antibody (1:1000, Millipore, USA) as secondary antibody and mounting with fluoroshield (Sigma-Aldrich, USA). Confocal micrographs were taken with Leica confocal scanning system mounted into a Leica TCS SP5 (Leica Microsystem GmbH, Mannheim, Germany), equipped with a 63X oil immersion objective and spatial resolution of approximately 200 nm in *x-y* and 100 nm in *z*. The 3D confocal scanning was performed by reconstructing the photoluminescence coming from different focalized slices with a sequential image acquisition. The optical sections were collected in transverse *x-z* and *y-z* planes.

Morphological characterization by atomic force microscopy (AFM).

The morphological characterization of isolated fluorescent microfibers by neuroblastoma cells was performed by tapping mode AFM using a Solver PRO Scanning Probe Microscope (NT-MDT Europe BV, Eindhoven, The Netherlands) in air at room temperature. We used TESPA (Veeco, Santa Barbara, CA) silicon cantilevers of 20–80 N/m spring constant and resonance frequency of around 300 kHz and scan size 5 μm x 5 μm.

Cellular adhesion.

Prior to microfibers deposition cover slip glass were cleaned with a piranha solution consisting of a 3:1 mixture of H_2SO_4 and H_2O_2, rinsed sequentially with deionized water, ethanol, acetone, and finally dried with nitrogen. 1 mg/mL of isolated fluorescent microfibers (Collagen I hybrid microfibers[11-12] and Vimentin hybrid microfibers) were deposited on cover slip glass and dried overnight. All the substrates were sterilized for 15 min using UV light before seeding cells.
Mouse myoblasts (C2C12), Mouse neuroblastoma cells (B104) and mouse embryonic fibroblasts (3T3) were seeded on microfibers at 100,000 cell/mL (approximately 1000 cells/mm^2 of substrate). After 48 hours each specimen was rinsed twice with PBS 1X to remove any unattached cells. Cytoskeleton morphology was investigated by phalloidin-TRITC at a final concentration of 1 mg/mL (Sigma-Aldrich) labeling. Cells were fixed in situ for 5 min in 3.7% formaldehyde, permeabilized with 1%Triton X-100 in PBS 1X, and washed again in PBS 1X. Thereafter, filamentous actin was stained with phalloidin-TRITC for 40 min at room temperature. Subsequently, the cells were washed several times with PBS to remove unbound phalloidin conjugate. The samples were examined using a Leica confocal scanning system mounted into a Leica TCS SP5 (Leica Microsystem GmbH, Mannheim, Germany) and equipped with a 63xoil immersion objective.

Proliferation assays.

Mouse myoblasts (C2C12), Mouse neuroblastoma cells (B104) and mouse embryonic fibroblasts (3T3) seeded on Collagen I hybrid microfibers and Vimentin hybrid microfibers were analyzed with the cytotoxicity test MTT, measuring the activity of living cells via mitochondrial dehydrogenase activity whose key component is 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide.[17] The controls were realized by cells culture on glass coverslip surfaces. Mitochondrial dehydrogenases of viable cells cleaves the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals are then dissolved in acidified isopropanol and the resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. B104 cells (100,000 cells/mL) were added to well culture plates at 1 mL/well and incubated at 37°C in 5% CO_2, 95% relative humidity for 24-48-72 and 192 hours with the DTTO dye suspension. The control was complete culture medium. After an appropriate incubation period, the cultures were removed from the incubator and the MTT solution added in an amount equal to 10% of the culture volume. Then the cultures were returned to incubator and incubated for 3 hours. After the incubation period, the cultures were removed from the incubator and the resulting MTT formazan crystals were dissolved with acidified isopropanol solution to an equal culture volume. The plates were read within 1 hour after adding acidified isopropanol solution. The absorbance was spectrophotometrically measured at wavelength 570 nm and the background absorbance measured at 690 nm substracted. The percentage viability is expressed as the relative growth rate (RGR) by equation:

$$RGR = (D_{sample}/ D_{control}) *100\%$$

where D_{sample} and $D_{control}$ are the absorbances of the sample and the negative control. Representative measurements of three distinct sets of data have been reported (Student t- test, $P < 0.05$).

Results and Discussion

Vimentin microfibers formation and characterization.

Live mouse neuroblastoma cells were incubated with micromolar DTTO in buffered solution (0.05 mg/mL). Following the removal of unbound fluorophore and extensive washing, the cells were continuously cultured and monitored for 7 days. **Figure 1** shows the LSCM images of the evolution with time of the neuroblastoma cells after incubation with DTTO. Thanks to the right hydrophilicity/hydrophobicity balance and the amphiphilicity due to the presence of the oxygen atoms, DTTO spontaneously crosses the cell membrane and diffuses into the cells. **Figure 1B** shows that after 1 h from washing the fluorophore is gathered inside the cells forming globular aggregates around the perinuclear region, as already observed for other thiophene fluorescent dyes.[10-12] After 24 h (**Figure 1C**) the cytoplasm of the cells appears intensely green fluorescent while the nuclei remain unstained and dark. Moreover, within the cytoplasm of several cells the formation of fluorescent microfibers of micrometer size is apparent. The microfibers progressively accumulate inside the cells (**Figure 1E-F**), while fluorescent cells are visible in the background indicating that fluorescence is transmitted from mother to daughter cells during the replication process. It is worth noting that many microfibers display details where helical green fluorescent structures are present, see for example the details shown in **Figure 1D** or **Figure 1S**.

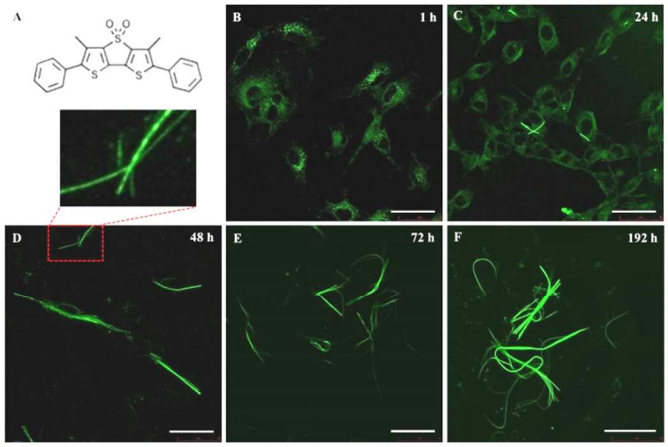

Figure 1. LSCM images of the intracellular formation of fluorescent microfibers in mouse neuroblastoma cells (B104 cell line). (A) Molecular structure of DTTO. (B-F) Time course of the cells incubated with DTTO at concentration of 0.05 mg/mL in DMEM medium. *Scale bars: 50 μm.*

The fluorescent microfibers formed after 7 days from treatment of the cells with DTTO were separated from cell lysate, picked up by LSCM and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The results are shown in **Figure 2**. SDS-PAGE unambiguously indicated the proteic nature of the fluorescent fibrils. Only one single signal was observed in the region around 60 KDa molecular weight.

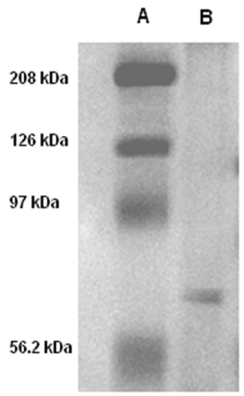

Figure 2. SDS-PAGE of the fluorescent microfibers isolated by live mouse neuroblastoma cells after 192 hours of treatment with DTTO (A= high molecular weight marker, B = sample).

The fluorescent microfibers isolated by neuroblastoma cells are mainly made of vimentin.

The fluorescent microfibers formed after 7 days from treatment with DTTO and separated from cell lysate were analyzed, after appropriate treatment (see Material & Methods section), by HPLC-ESI-QToF using the Mascot software[18] for proteins identification. It was found that the 73.4% (See supplementary attached file denominated 'Fibrille_Rattus') of the fluorescent microfibers was made of vimentin, a protein of the intermediate filaments of the cytoskeleton with a 57 KDa molecular weight.[19] A few percent of nestin, plectin and lamin A were also detected (See again the file 'Fibrille_Rattus'). The presence of the latter proteins is in agreement with the fact that vimentin presents a subcellular distribution and interaction with many proteins as lamin, plectin, etc.. In particular, at the nuclear envelope, the 6.6 kDa tail region of vimentin is known to interact with lamin B,[20] while another study shows that vimentin interacts with β3 integrins and plectin, which together regulate the organization and distribution of vimentin in several cell types.[21]

In agreement with mass spectrometry data, co-localization experiments of isolated fluorescent microfibers with the monoclonal vimentin antibody showed that there was in fact co-localization between the antibody and the microfibers. **Figure 3** shows the LSCM images of isolated green fluorescent microfibers (a), isolated red fluorescent anti-vimentin antibody (b), merge images (c) and corresponding z-stack sections (d) with z-resolution of 200 nm. **Figure 3D** shows that there is spatial co-localization with the red fluorescent anti-vimentin antibody inside the green fluorescent microfibers (See supplementary attached file denominated 'LCSM_zeta stack sections of vimentin co-localization').

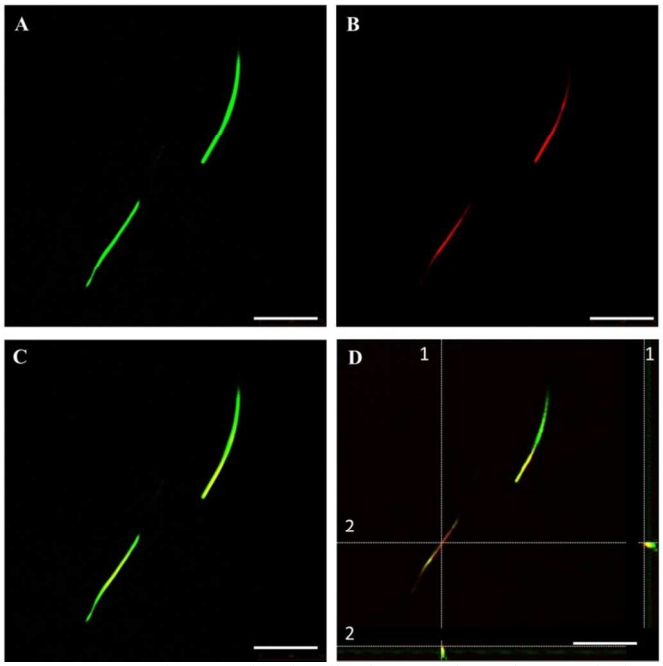

Figure 3. LSCM images of co-localization experiment between isolated fluorescent microfibers secreted by B104 mouse neuroblastoma cells and anti-vimentin antibody. A) Isolated green fluorescent microfibers. B) Isolated red fluorescent anti-vimentin antibody. C) Merge image. D) Corresponding z-stack sections with z-resolution of 200 nm, showing the spatial co-localization of DTTO (green) with anti-vimentin antibody (red) inside the microfibers. *Scale bar: 7.5 μm*

Figure 4 shows the AFM image of fluorescent microfibers isolated from the cell lysate. The morphology of the largest fibers recalls the structure of the dimer of vimentin representing the first level of vimentin self-assembly.[22-26] The figure also displays the coiled coil arrangement of two smaller microfibers. **Figure 2S** shows another fluorescent microfiber isolated from cell lysate and provides evidence of the lateral growth of the microfibers.

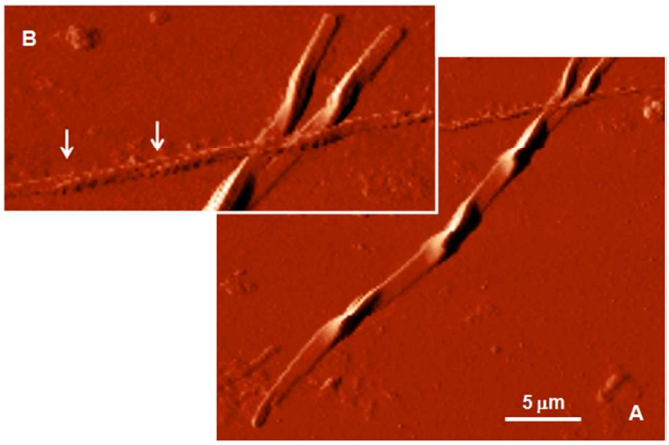

Figure 4. A) AFM image of fluorescent microfibers isolated from the cellular milieu. The morphology of the largest fiber recalls the structure of the dimer of vimentin representing the first level of vimentin self-assembly.[22] B) Magnification of a detail of Figure A. The arrows indicate the coiled coil arrangement of two smaller microfibers.

Vimentin is a polypeptide comprising 466 amino acids with a highly conserved α-helical 'rod' domain that is flanked by non- α-helical N-

Journal of Materials Chemistry B RSCPublishing

ARTICLE

and C-terminal end domains, the 'head' domain and 'tail' domain, respectively.[13] The self-organization of these molecules leads to the formation of coiled coils, which are the basic structural building blocks for the entire IF family of proteins. Vimentin is also known to form homopolymers and heteropolymers (i.e., it associates with other type III and type IV IFs), a common feature among the members of the IF family ascribed to the presence of a coiled-coil α-helical domain. Highly stable polymers are formed, the stability of which is controlled by the phosphorylation status of the integral proteins. The astonishing similarity between the sophisticated shape of the fluorescent microfiber analyzed by AFM in **Figure 4** and the shape of human vimentin dimer as determined on the basis of X-ray diffraction at the atomic scale (see figure 4C of reference 26) is unlikely to be the outcome of a mere coincidence. The fact that cell viability remains unaltered upon DTTO uptake (**Figure 3S**) plaids in favor of the fact that the fluorescent dye is progressively embedded during the protein growth without substantially altering the self-assembly process. DTTO is a small neutral molecule which can easily be accommodated within the alpha helical collagen strand of fibroblasts of different origin via hydrogen bonds leading to the stabilization of the helical structure.[11-12] All IF proteins, including vimentin, although varying greatly in their primary structure, are characterized by an alpha-helical central rod domain having the intrinsic capability to form coiled-coil filaments and are terminated by non-helical N- and C-domains of variable size, which play a role in protein self-assembly process. Thus, the α-helical structure present in vimentin and its ability to form non-covalent bonds with DTTO via non-bonding interactions cannot alone justify the fact that it is mainly vimentin that is concerned by DTTO in live neuroblastoma cells. It is more likely that the choice is determined by some co-factors related to the general 'economy' of the cell and its metabolism.

Cell interaction with collagen-DTTO and vimentin-DTTO microfibers.

The possibility to separate by LSCM the physiologically formed microfibers from cell lysate thanks to their fluorescence offers an unprecedented way to test their potentialities as innovative biomaterials. Here, we describe initial experiments to test their effect on various live cell lines.

As shown in **Figure 5**, cells seeded on collagen-DTTO or vimentin-DTTO microfibers assumed different morphologies and configurations depending on the stimuli experimented.

After having been seeded on collagen-DTTO microfibers for 48 hours, C2C12 myoblasts and B104 neuroblastoma cells (**Figures 5Ae, 5Ag**) showed cytoskeleton remodeling, with a remarkable organization of F-actin into intricate branching networks and polarized filopodia protrusions associated to a more elongated shape, whereas 3T3 fibroblasts (**Figure 5Af**) assumed a spindle-shaped appearance displaying a round, contracted cell body. Conversely, when seeded for 48 hours on vimentin-DTTO microfibers, 3T3 fibroblasts (**Figure 5Bf**) and B104 neuroblastoma cells (**Figure 5Bg**) maintained their epithelial morphology of a polygonal shape with many actin cytoplasmic protrusions (filopodia-like processes), while C2C12 myoblasts showed a contracted cell body. As shown in the figure, after 48 h of cell culture, collagen-DTTO and vimentin-DTTO microfibers appeared as green fluorescent dots, with uniform distribution inside the cytoplasm of C2C12 myoblasts (**Figures 5Ab, 5Bb**) and 3T3 fibroblasts (**Figures 5Ac, 5Bc**). In this case it can be guessed that both types of microfibers were degraded by the cell,[27,28] conceivably by action of the matrix metalloproteases modifying in this way the microenvironment and resulting in alteration of cellular morphology and cytoskeleton rearrangement. On the contrary, in B104 neuroblastoma cells collagen-DTTO microfibers were partially degraded, as evident in **Figure 5Ad**, while vimentin-DTTO microfibers show their native morphology (**Figure 5Bd**). The degradation and possible recycling of the microfibers can be a process used to create protein fragments needed to build new cellular structures.

Journal of Materials Chemistry B RSCPublishing

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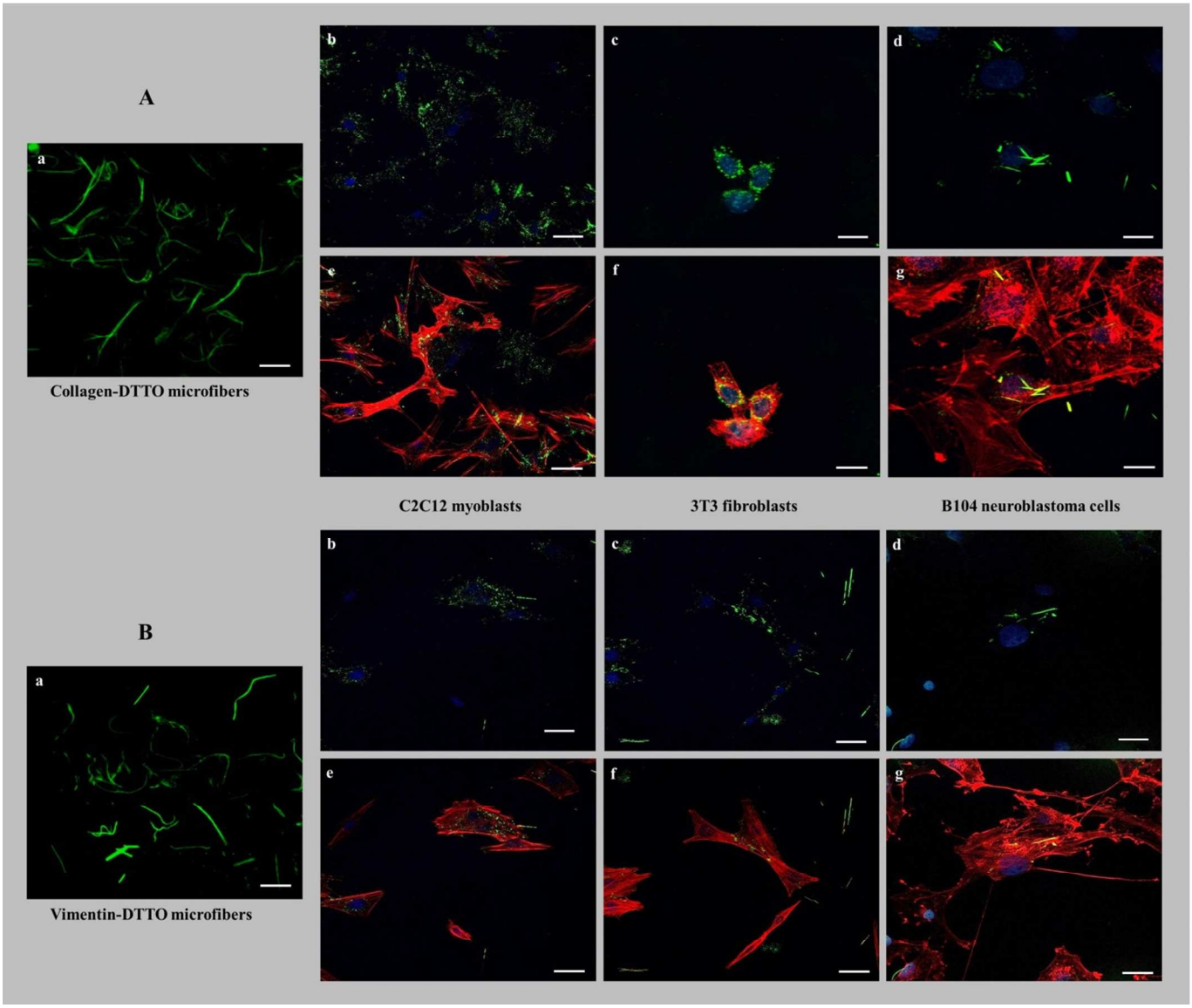

Figure 5. **Aa, Ba)** LSCM images of pristine collagen-DTTO and vimentin-DTTO microfibers used as substrates for the deposition of live C2C12 myoblasts, 3T3 fibroblasts and B104 neuroblastoma cells. **A**) LSCM images of fixed C2C12 myoblasts (b,e), 3T3 fibroblasts (c,f) and B104 neuroblastoma cells (d,g) seeded on collagen-DTTO microfibers for 48 hours, stained with DAPI (blue, b-d) and actin (red, e-g). **B**) The same for vimentin-collagen microfibers. *Scale bar: 10 µm*

We also evaluated the viability of C2C12 myoblasts, 3T3 fibroblasts and B104 neuroblastoma cells cultured on a glass cover slip coated with collagen-DTTO or vimentin-DTTO microfibers by performing a MTT proliferation assay at 24, 48, 72, 192 h. As shown in **Figure 6**, substrates coated with vimentin microfibers did not affect cell viability or proliferation, as judged by the increases in total cell numbers over time. The same was true for myoblasts and neuroblastoma cells coated on collagen-DTTO microfibers. On the contrary, the viability of 3T3 fibroblasts was compromised when the cells were seeded on collagen-DTTO microfibers. As evident in **Figure 5** all microfibers (1 mg/mL) present on the substrate were degraded and internalized by fibroblasts after 48 h. These results indicate that the massive storage of collagen-DTTO fragments inside fibroblasts leads to apoptosis. This does not exclude that concentrations below 1 mg/mL of

collagen-DTTO microfibers might not induce cytotoxic effects on fibroblasts.

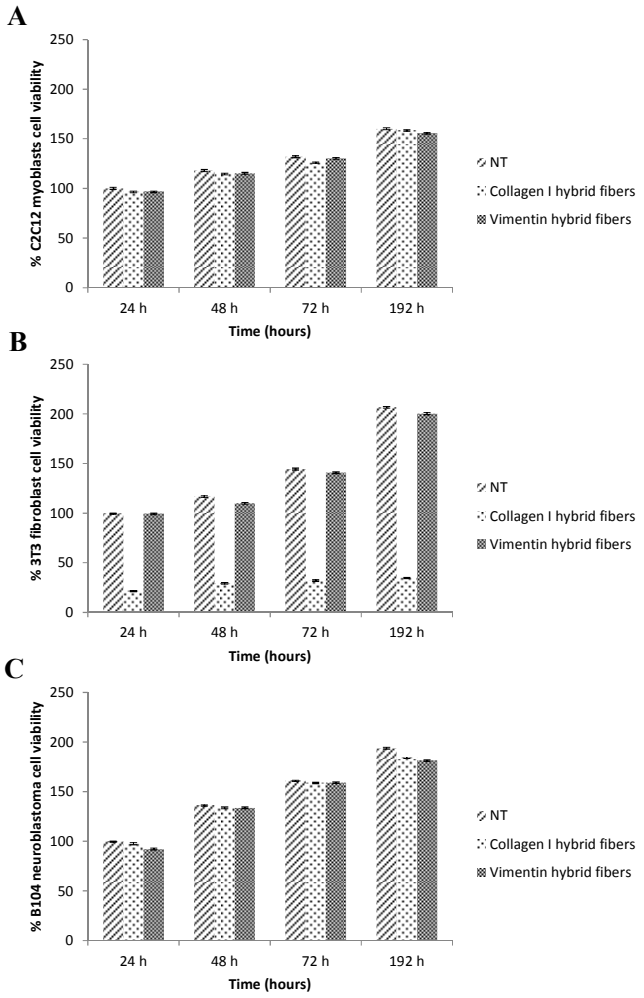

Figure 6. Proliferation assays of C2C12 myoblasts (A), 3T3 fibroblasts (B) and B104 neuroblastoma cells (C) cultured on a uncoated glass cover slip (NT), glass cover slip coated with collagen I or vimentin hybrid microfibers for 24,48,72,192 h. The results are expressed as mean ± standard deviations and are representative measurements of three distinct sets of data. No significant difference between values at different time points is observed at $P < 0.05$ with *t-Student test*.

Further experiments are in progress to elucidate the biochemical mechanisms involved in the degradation of the fluorescent microfibers and to get information on how their mechanical and electrical characteristics direct the cellular behaviour.

Conclusions

The results reported here demonstrate that different types of cells employ the organic dithienothiophene fluorophore DTTO for the physiological production of fluorescent fibrillar proteins organized in nanostructured microfibres, namely vimentin with neuroblastoma cells (this study) and type-I collagen with fibroblasts of different origin (reference 11). Moreover, various types of live cells seeded on these microfibers are able to internalize and degrade them, experiencing in turn different effects on their morphology and viability.

The composition and distribution of specific extracellular matrix components vary with the type of tissue, and can also be altered by tissue's development stages and/or pathological state. Cells sourced from different tissue typically yield matrices that mimic the relative composition of the natural tissue matrix. In this paper, we demonstrate that the cell source is the primary determinant of the resulting multiscale hybrid microfibers, in particular we used live neuroblastoma cells that produce hybrid vimentin microfibers upon uptake of DTTO. The different composition induces dissimilar cellular behaviour when these multiscale microfibers are used as substrates for cell cultures.
The requirements for materials used in biomedical applications are biocompatibility and biodegradability. The fluorescent microfibers physiologically secreted by live cells described here present both characteristics. These microfibers represent innovative multiscale biomaterials useful not only for the imaging of cellular processes but potentially also for drug delivery and tissue engineering as well as to confer bioactivity to synthetic scaffolds through the loading of bioactive molecules as growth factors.

Notes and references

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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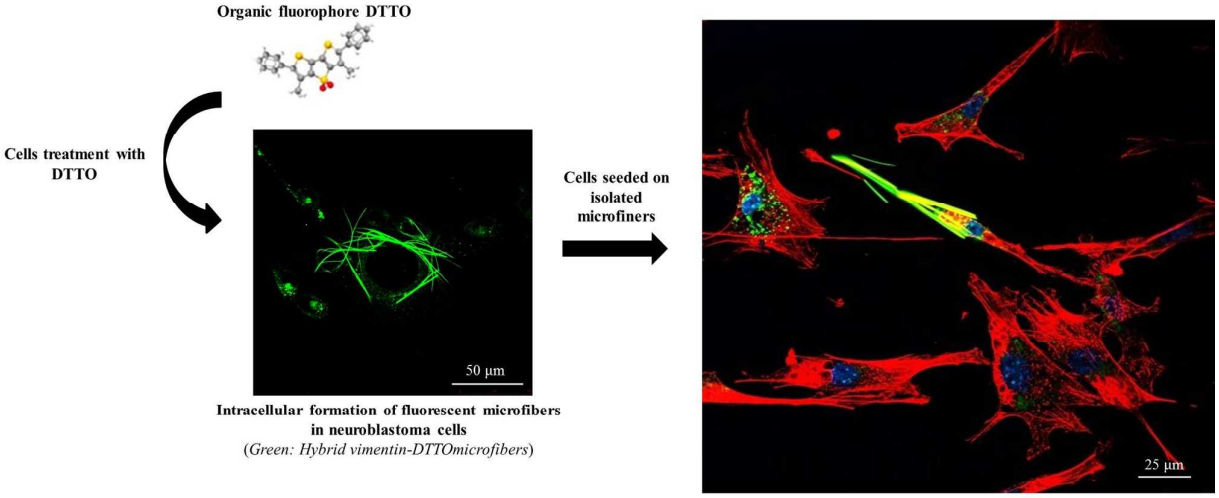
Physiological secretion of fluorescent nanostructured microfibers upon spontaneous uptake of the appropriate organic fluorophore by live cells and effects of cells seeding on isolated microfibers.



Intracellular formation of fluorescent microfibers in neuroblastoma cells
(*Green: Hybrid vimentin-DTTOmicrofibers*)

Myoblasts seeded on hybrid vimentin-DTTO microfibers
(*Red: Actin; Green: vimentin-DTTO microfibers; Blue: DAPI*)