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ARTICLE TYPE

SUPPLEMENTARY MATERIAL

Sulphonic groups induce formation of filopodia in mesenchymal stem cells

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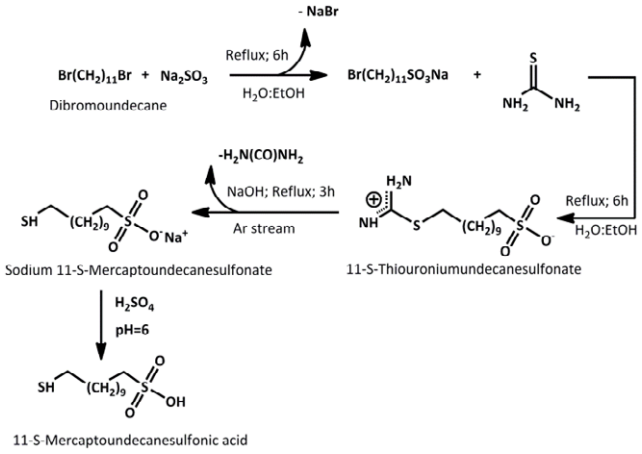
Preparation and characterisation of 11-S-mercapto undecane sulphonate

11-mercapto-1-undecanol (97%), 1,11-dibromoundecane (98%), sodium sulphite (98%) and thiourea (99%) were purchased from Sigma-Aldrich and used without further purification. 11-mercaptoundecanesulphonic acid was synthesised adapting previously reported procedure (Scheme 1).



Scheme 1. Synthesis of 11-mercaptoundecanesulphonic acid

Synthesis of 11-bromoundecane sulphonate (sodium salt)

1,11-dibromoundecane (11.58mL, 48.2mmol) was mixed with 300mL of ethanol and 50mL of deionized water and the mixture was heated until refluxing. Sodium sulphite (1.8g, 14.2mmol) was solubilised in 50mL of water and added stepwise (during 2.5h) to the refluxing mixture. The reaction medium was allowed to reflux for an extra 3.5h. The mixture was transferred to a separation funnel and 100mL of deionized water was added. The unreacted 1,11-dibromoundecane (lower phase) was separated from the mixture and the top phase was washed three times with petroleum ether. The aqueous phase was concentrated to 50mL under reduced pressure, and the 11-bromoundecane sulphonate (50-60% yield) was crystallized from the solution and collected by vacuum filtration. ^{1}H NMR (300MHz, D_2O): δ 3.54 (t, 2H, $BrCH_2$), 2.90 (m, 2H, CH_2SO_3Na), 1.87 (m, 2H, $BrCH_2CH_2$), 1.73 (m, 2H, $CH_2CH_2SO_3Na$), 1.42 (m, 4H, CH_2), 1.31 (m, 10H, CH_2). Elemental analysis (found/calculated): C, 41.4/39.2;H, 7.6/6.6.

Synthesis of thiouronium undecane sulphonate

11-bromoundecane sulphonate (1.13g, 3.3mmol) from the previous step and thiourea (0.26g, 3.3mmol) were solubilised in a 1:1 mixture of water:ethanol (80mL) and refluxed for 6h. The solution was filtered and the ethanol was removed by vacuum evaporation. The aqueous solution was cooled and the white precipitate was collected by vacuum filtration (and twice washed with deionised water). Thiouronium undecane sulphonate was collected upon recrystallisation in deionised water (60-70% yield). ^{1}H NMR (300MHz, DMSO-d_6): δ 8.97 (s, 4H, NH_2), 3.38 (s, 2H, SCH_2), 2.49 (t, 2H, CH_2SO_3Na), 1.61 (m, 4H, CH_2) 1.27 (m, 14H, CH_2). Elemental analysis (found/calculated): C, 47.7/46.4; H, 9.0/8.4.

Synthesis of 11-S-mercapto undecane sulphonate

Thiouronium undecane sulphonate (0.2g, 0.6mmol) was added to an aqueous solution of sodium hydroxide (10mL, 10% m/v). The mixture was refluxed for 3h under Ar atmosphere and was allowed to cool to room temperature forming a white precipitate. The mixture was neutralized with sulphuric acid (until pH≅6) and cooled on ice. The white precipitate was collected by vacuum filtration and dried overnight in a vacuum oven. The 11-S-mercapto undecane sulphonate (30-40% yield) was collected after recrystallization from ethanol (under Ar atmosphere) and characterised (Fig. 1). ^{1}H NMR (300MHz, D_2O): δ 2.90 (t, 2H, CH_2SO_3Na), 2.56 (t, 2H, $HSCH_2$), 1.73 (m, 2H, $CH_2CH_2SO_3Na$), 1.61 (m, 2H, $HSCH_2CH_2$), 1.4-1.2 (m, 14H, CH_2). ESI: 267.1 $(M\text{-}Na)^-$.Elemental analysis (found/calculated): C, 45.2/45.4; H, 8.1/8.0.

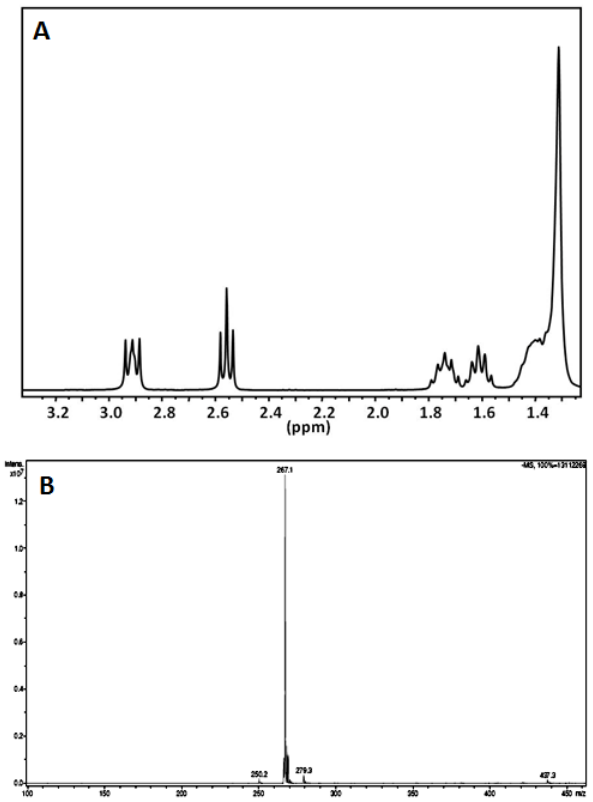


Figure 1. ^{1}H-NMR (A) and electrospray ionisation mass spectrometry (ESI-MS) (B) of 11-S-mercapto undecane sulphonate

Isolation and expansion of human mesenchymal stem cells

Human bone marrow derived mesenchymal stem cells (BM-MSCs)

Human bone marrow aspirates were obtained from healthy patients under the scope of a cooperation agreement with Hospital da Prelada (Porto, Portugal). Bone marrow mononucleated cells (BMMNCs) were separated on a Histopaque density gradient (1.077g/mL, Sigma-Aldrich) and washed with isotonic Phosphate Buffered Saline solution (PBS, Sigma-Aldrich). BMMNCs were expanded in α-modified Eagle's medium (α-MEM, Sigma-Aldrich) supplemented with 1% Antibiotic/Antimycotic (Gibco), 10% fetal bovine serum (FBS, Gibco) and 2ng/mL bFGF.

Adipose tissue digestion and cell harvesting

Human subcutaneous adipose tissue samples (age range of 20-36 years) were obtained from lipoaspiration procedures under the scope of a cooperation agreement with Hospital da Prelada (Porto, Portugal). The adipose tissue was washed with PBS containing 10% Antibiotic/Antimycotic and then digested with a 0.1% collagenase from *Clostridium histolyticum* (Sigma-Aldrich) solution in PBS for 45min at 37°C under gentle stirring. The digested tissue was gently pressed through a strainer and centrifuged at 1000 *g* for 10min. The cell pellet was resuspended and incubated in lysis buffer (155mM NH_4Cl, 5.7mM K_2HPO_4, 0.1mM EDTA) 10min before centrifugation at 800 *g* for 10 min. Cells were expanded in α-modified Eagle's medium (Sigma-Aldrich) supplemented with 1% Antibiotic/Antimycotic (Gibco), 10% FBS (Gibco).

Formation and characterisation of SAMs

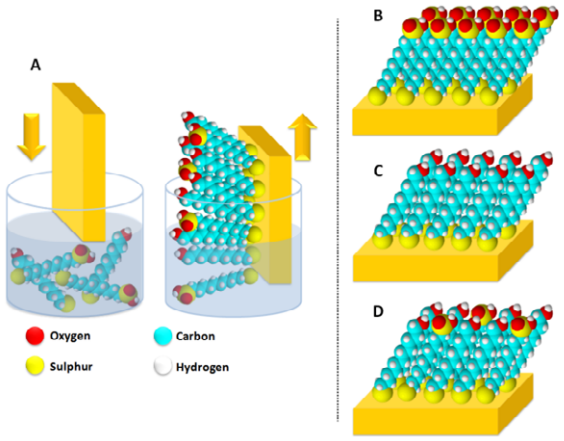


Figure 2. Schematic presentation of the formation of SAMs on a gold surface (A) and the obtained surfaces SO3H 100 (B), SO3H 0 (C) and mixed (SO3H 25, SO3H 75) SAMs (D)

Table 1. XPS data for the obtained SAMs

Sample	Elemental composition [at%]			Binding Energy [eV]	
	C	O	S	C1s	S2p
SO3H 0	81.1	10.5	8.4	285[1] (79.6%) 286.3[2] (20.4%)	162.3[3](100%)
SO3H 25	82.2	6.6	9.9	285 (74.5%) 286.3 (25.5%)	162.3 (79.4%) 169.7[4] (20.6%)
SO3H 75	80.2	13.3	6.5	285 (81.2%) 286.3 (18.8%)	162.2 (43.7%) 163.4 (26.4%) 168.6 (29.9%)
SO3H 100	70.8	22.8	6.4	285 (82.5%) 286.2 (10%) 287.97 (7.5%)	162.2 (47.4%) 163.47 (8.1%) 168.44 (52.6%)

[1]C-C, C-H bound; [2]C-OH bound; [3]thiol-bound to Au; [4]oxidised sulphur

Effect of surface chemistry on cell adhesion, spreading and cytoskeleton organisation

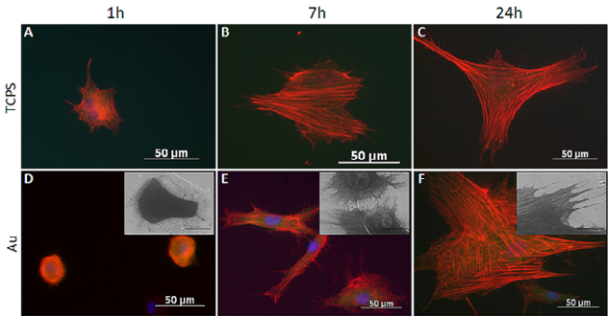


Figure 3. Fluorescence microscopy images of BM-MSCs cultured on control surfaces TCPS (A-C) and Au (D-F): focal adhesions formation was evaluated by immunostaining of vinculin (green); cytoskeleton organisation through fluorescent staining of actin (red) and nuclei were counterstained with DAPI (blue). The insets correspond to SEM micrographs of the same samples (bars = 10 µm).

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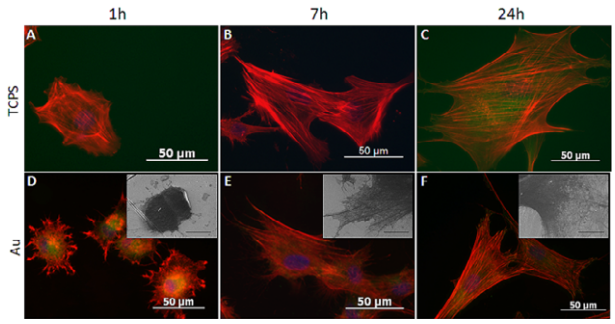

Figure 4. Fluorescence microscopy images of ASCs cultured on control surfaces TCPS (A-C) and Au (D-F): immunostaining of vinculin (green), actin (red) and nuclei (DAPI, blue). The insets correspond to SEM micrographs of the same samples (bars = 10 µm).

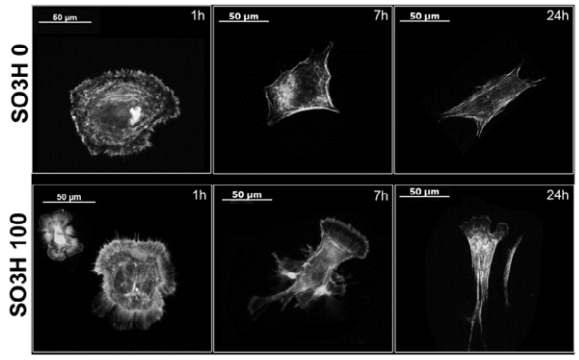

Figure 5. Assembly and organisation of the actin cytoskeleton of BM-MSCs cultured on SO3H 0 (up) and SO3H 100 (down) surfaces.

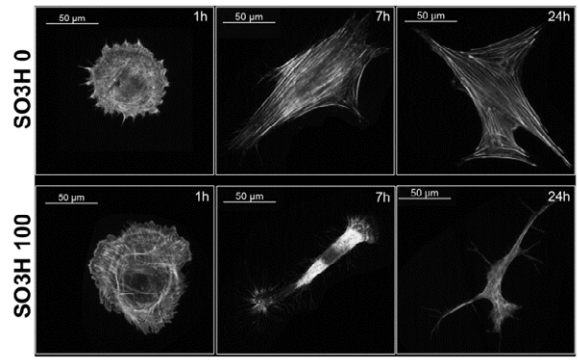

Figure 6. Assembly and organisation of the actin cytoskeleton of ASCs cultured on surfaces functionalised with –OH and –SO3H groups.

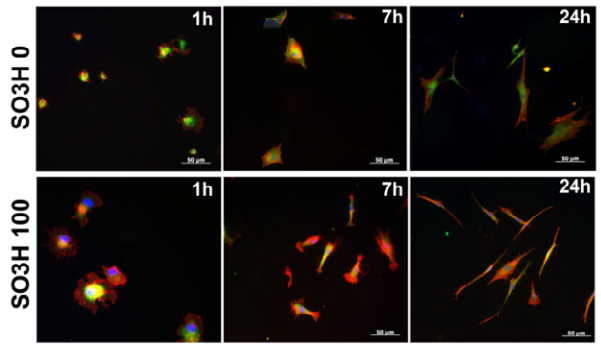

Figure 7. Representative fluorescence microscopy images of BM-MSCs cultured on $HS(CH_2)_{11}OH$ and $HS(CH_2)_{11}SO_3H$ SAMs.

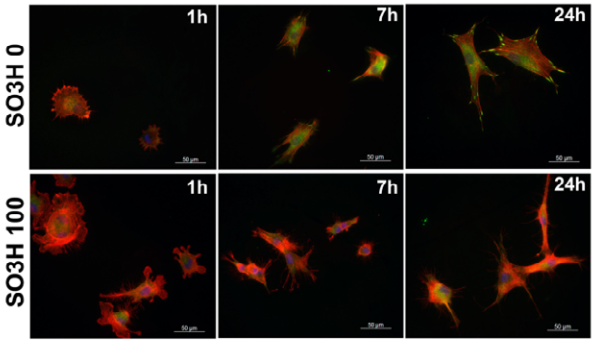

Figure 8. Representative fluorescence microscopy images of ASCs cultured on $HS(CH_2)_{11}OH$ and $HS(CH_2)_{11}SO_3H$ SAMs.