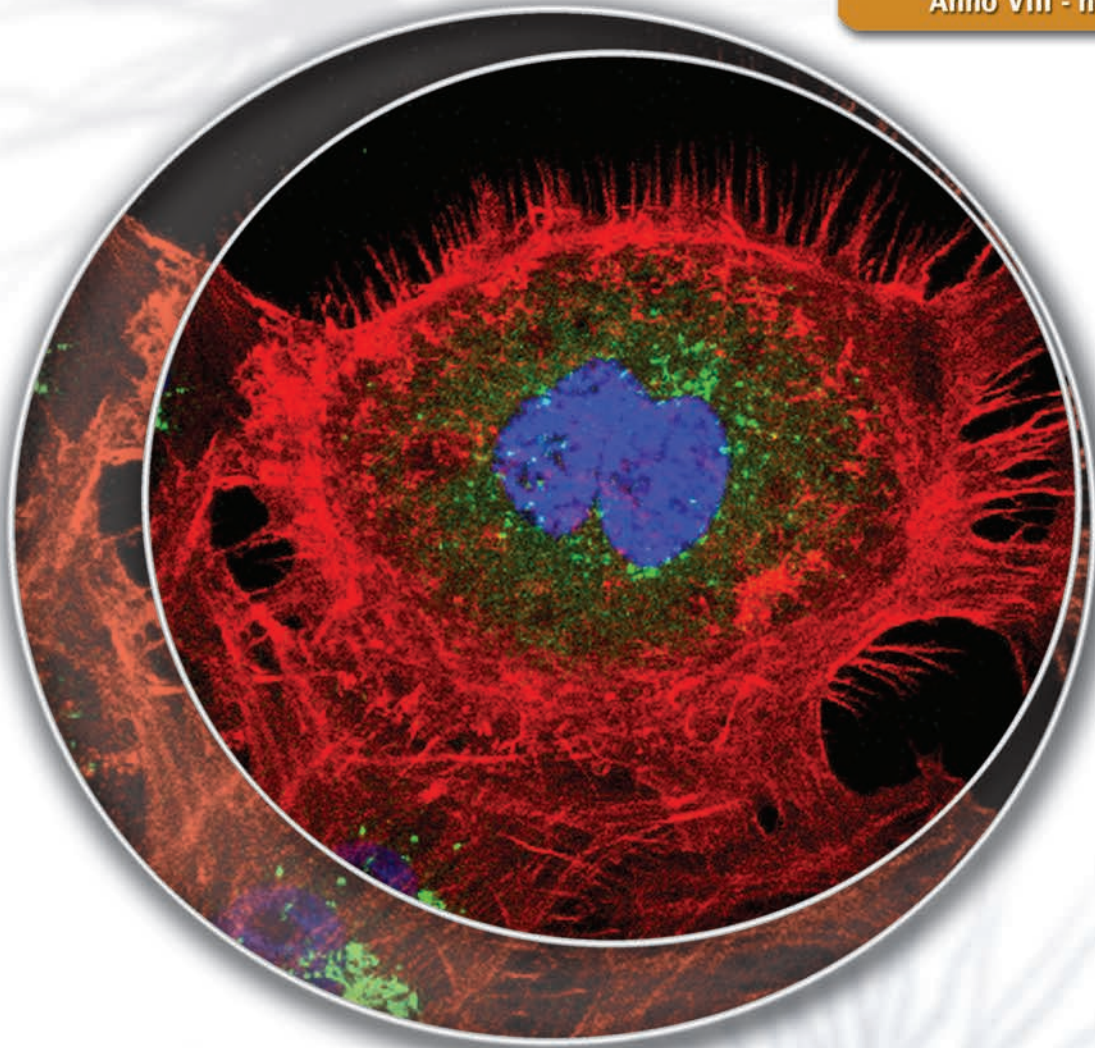


microscopie

Anno VIII - n. 1 (15) - Marzo 2011

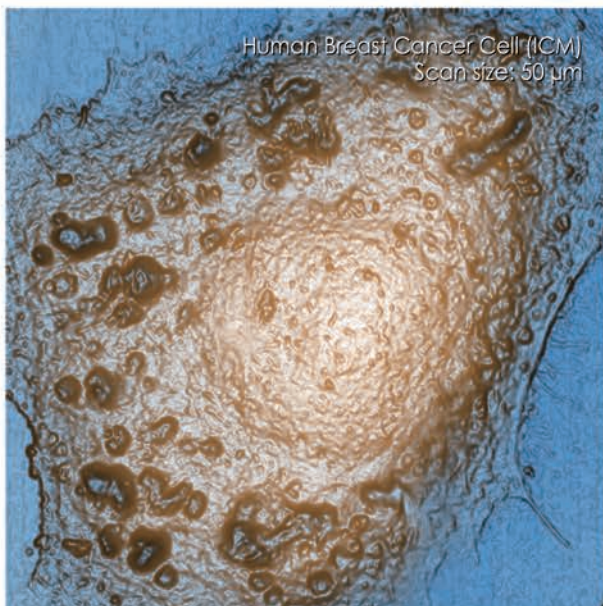


Attività SISM 2011
MCM 2011
Premio "Carla Milanese"
e Contributi di partecipazione al MCM 2011
Centro Grandi Strumenti (Università di Pavia)



**Società Italiana
Scienze Microscopiche**

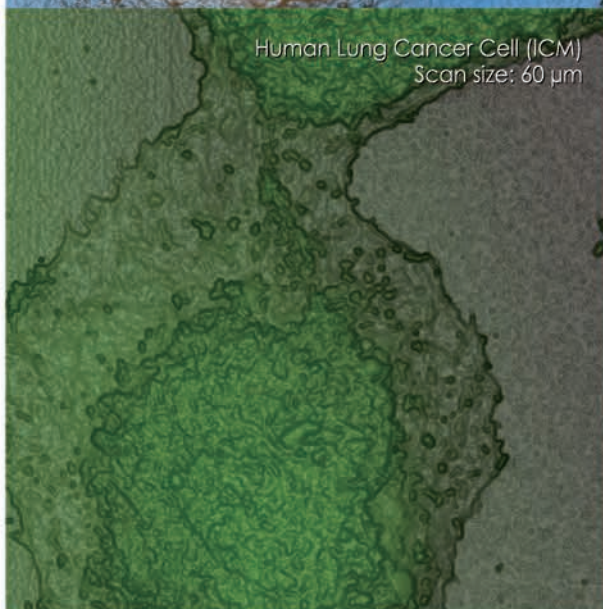
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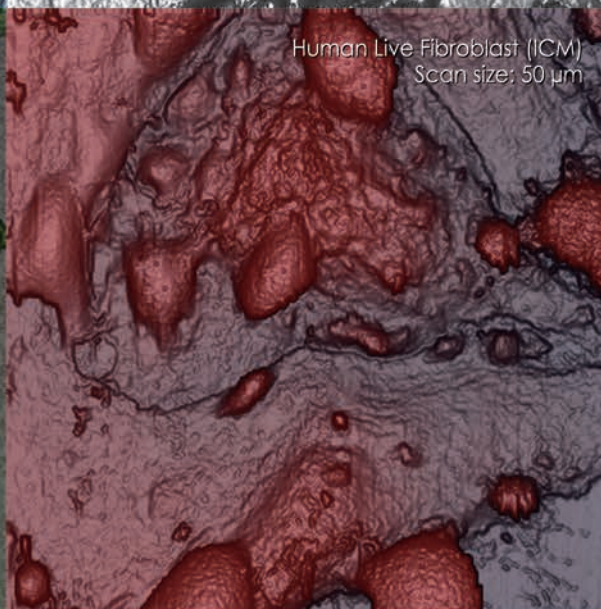
Human Breast Cancer Cell (ICM)
Scan size: 50 μm



Chromosomes (AFM)
Scan size: 20 μm



Human Lung Cancer Cell (ICM)
Scan size: 60 μm



Human Live Fibroblast (ICM)
Scan size: 50 μm



S-Layer (AFM)
Scan size: 500 nm

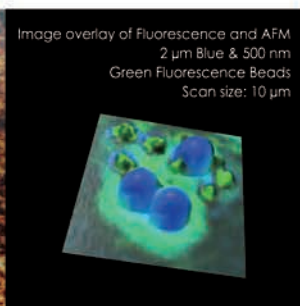


Image overlay of Fluorescence and AFM
2 μm Blue & 500 nm
Green Fluorescence Beads
Scan size: 10 μm



STO-feeder-cells
Scan size: 150 μm



Human Fibroblast Cell
Scan size: 50 μm

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Microscopiche
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Aut. Trib. n. 688 S.P. del 26 marzo 2008

In copertina: Cellule HeLa: immunomarcatura dell'apparato
di Golgi (verde) e rivelazione dell'actina con falloidina
(rosso); colorazione del DNA con Hoechst 33258.
L'immagine a microscopia confocale è stata acquisita presso
il Centro Grandi Strumenti dell'Università di Pavia
(cortesia di M.G. Bottone).

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ISCRIZIONE

Possono iscriversi alla Società i ricercatori e gli operatori professionali comunque attivi nel campo delle diverse microscopie. Per l'iscrizione alla Società è necessario compilare la richiesta di associazione ed inviarla al Presidente. La scheda di associazione può essere compilata direttamente sul sito web della società all'indirizzo www.sism.it oppure può essere reperita in questo periodico ed inviata via fax. Le richieste verranno valutate dal Consiglio Direttivo nella prima riunione utile e l'approvazione dei nuovi Soci sarà comunicata personalmente agli interessati. Dopo tale comunicazione il nuovo socio può procedere al pagamento della quota sociale secondo le modalità riportate sotto.

QUOTA SOCIALE

La quota sociale è di € 35 per i soci ordinari e di € 25 per i non strutturati. I soci non strutturati, unitamente alla quota sociale, dovranno far pervenire al Presidente della Società una dichiarazione attestante il proprio status.

Modalità di pagamento:

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- mediante invio di un assegno bancario non trasferibile intestato a S.I.S.M.
l'assegno deve essere spedito alla Dott.ssa Amelia Montone, ENEA, Dipartimento Tecnologie Fisiche e Nuovi Materiali, C.R. Casaccia, Via Anguillarese, 301 - 00123 Roma
- mediante bonifico bancario intestato a S.I.S.M.
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E-mail: amelia.montone@enea.it
P.IVA 05089821002 C.F. 80181630155

Si ricorda che le richieste di associazione verranno valutate dal Consiglio Direttivo e l'approvazione dei nuovi Soci verrà comunicata personalmente agli interessati.

Il pagamento della quota di associazione deve essere effettuato solo dopo il ricevimento della comunicazione dell'approvazione, da parte del Direttivo, della richiesta di associazione.

Il sottoscritto rischiede l'ammissione alla SISM in qualità di:

- Socio ordinario (35 euro)
 Socio non strutturato (25 euro)

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Data di nascita

Titolo di studio e qualifica

Tipo di istituzione

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Istituto/Ente/Ditta

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Settore di attività

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Come deliberato nell'Assemblea Generale del 24/09/2001 ogni Socio SISM è anche Socio EMS.

Questi stessi dati saranno pertanto automaticamente inviati anche all'EMS, di cui la SISM fa parte. I dati dei Soci sono utilizzati dalla Segreteria EMS per distribuire il Notiziario in forma elettronica, per annunciare informazioni importanti come Congressi, Corsi, Scuole e per pubblicare l'Annuario dei Soci EMS.

Se si desidera che i propri dati personali non compaiano nell'annuario EMS, selezionare l'apposita opzione.

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Editoriale

Cari Amici,

siamo in piena preparazione del MCM 2011, già molti invited speaker, individuati dall'International Advisory Board, hanno accettato di venire al 10th Multinational Congress on Microscopy 2011 ad Urbino dal 4 al 9 settembre 2011 ed al Workshop satellite "Tomography, 3D Reconstruction and 3D Imaging Techniques" che si terrà ad Ancona il 3 e il 4 settembre 2011. Sul sito del Congresso (<http://www.mcm2011urbino.it>), troverete importanti aggiornamenti, sono aperte le registrazioni e le sottomissioni degli abstracts, aspettiamo le vostre proposte! Elisabetta Falcieri, Presidente del Congresso, ci aggiornerà su tutti gli aspetti del congresso all'interno della Rivista.

La SISM, in collaborazione con le Ditte che ci supportano, ha bandito i Contributi di Partecipazione ed il Premio Carla Milanese per il MCM 2011.

Per il 2011 è previsto il Congresso biennale della SISM e l'assemblea ordinaria dei soci SISM, che si svolgeranno all'interno del MCM 2011. Durante l'assemblea avverrà la designazione dei candidati per il rinnovo del Consiglio Direttivo; ricordo che solo chi è in regola con le quote associative ha diritto alla candidatura ed alle votazioni che seguiranno.

Sono ancora pochi i Soci in regola con il pagamento delle quote associative, vi ricordo che i Soci morosi da oltre due anni sono considerati decaduti dalla Società e questo comporta la cancellazione dall'elenco dei Soci SISM e dall'elenco dei Soci EMS, vi prego quindi di mettervi in regola al più presto per continuare ad essere parte attiva della SISM.

Per quanto riguarda le iniziative nazionali della SISM per il 2011, quest'anno saranno organizzati tre eventi:

- Scuola teorico-pratica di microscopia elettronica a trasmissione ed elaborazione di immagini - Livello base a Modena, C.I.G.S. (Università degli Studi di Modena e Reggio Emilia) dal 15 al 17 giugno 2011
- Scuola teorico-pratica di Microscopia Elettronica a Scansione in Scienza dei Materiali a Roma, ENEA C.R. Casaccia, dal 4 al 6 ottobre 2011
- Scuola Avanzata di Microscopia a Scansione di Sonda a Bologna, CNR-ISMN, ISOF, dal 28 novembre al 3 dicembre 2011.

Per gli aggiornamenti sulle attività SISM vi invito a consultare il nostro sito web (<http://www.sism.it>).

In questo numero di Microscopie sono stati pubblicati i lavori dei vincitori del Premio SISM 2010, ci aspettiamo ad Urbino contributi di simile livello da parte dei giovani ricercatori italiani.

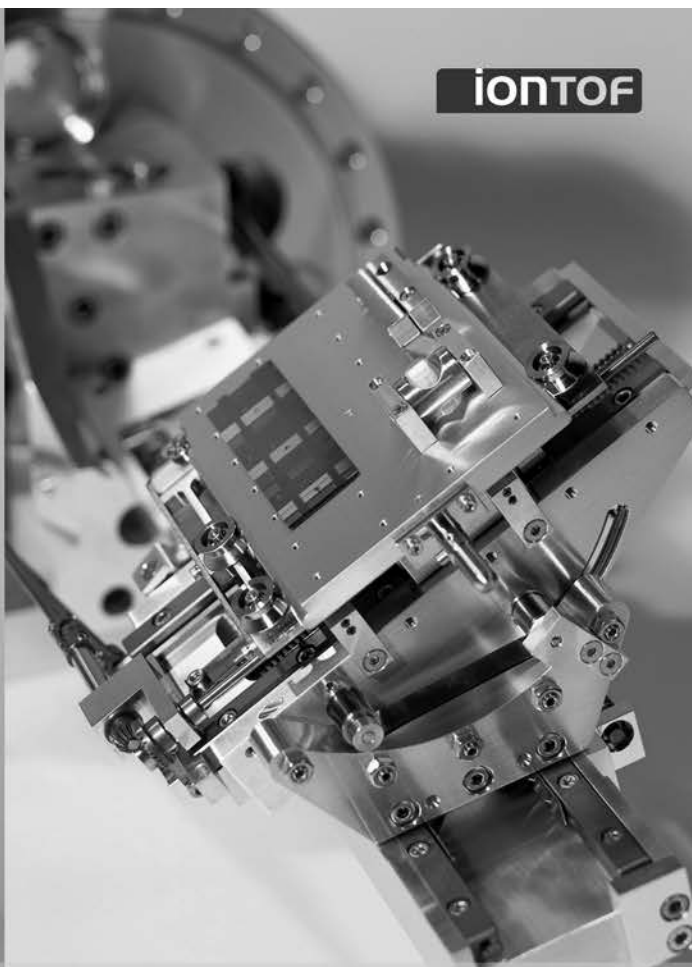
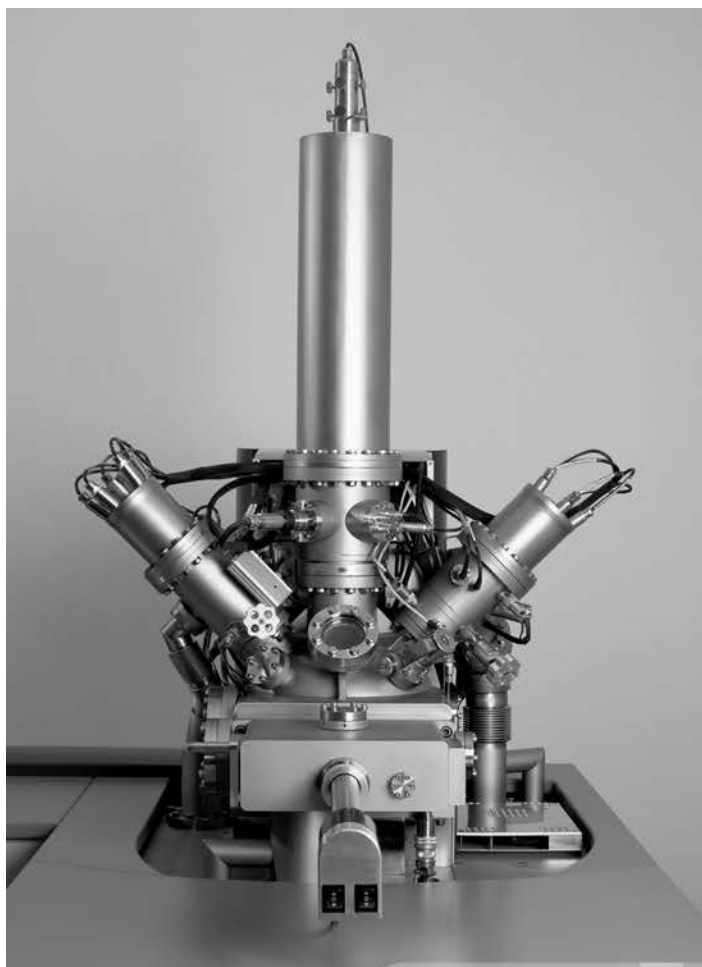
La SISM, come sempre, rivolge particolare attenzione ai giovani con le attività di formazione, con i Contributi ed i Premi per la partecipazione al Congresso e con quote di iscrizione ridotte agli eventi SISM ed al MCM 2011.

Buon lavoro!

Amelia Montone



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Editoriale

Cari Soci,

quello che è appena cominciato è un anno particolarmente importante per tutti noi. Come ben sapete, in settembre 2011 si terrà a Urbino il Multinational Congress on Microscopy, organizzato per la SISM da Elisabetta Falcieri. Si tratta di una straordinaria occasione per la nostra Società e per noi Italiani di dimostrare, ancora una volta, le capacità organizzative e di accoglienza di cui siamo capaci. Il mio auspicio è che ogni Socio contribuisca al successo del Congresso non solo partecipando in prima persona, a dimostrare la validità della ricerca scientifica Italiana, ma anche svolgendo azione promozionale presso i propri colleghi ed amici, così da assicurare un'ampia adesione dei nostri microscopisti.

Un piccolo contributo lo dà anche Microscopie, dedicando ampio spazio, in questo numero, al MCM 2011. Elisabetta Falcieri ha indirizzato una lettera ai Soci SISM, e vengono anche riportate tutte le informazioni al momento disponibili sulle tematiche scientifiche del Congresso, le possibilità logistiche e le opportunità turistiche offerte dalla splendida Urbino e dal Montefeltro. Certamente questa azione promozionale sarà apprezzata e solleciterà nei nostri Soci il desiderio di mostrare i risultati delle loro ricerche in uno stimolante contesto internazionale, insieme alla piacevole curiosità di conoscere (o rivisitare) una Regione affascinante.

In questo numero vengono anche descritte le iniziative didattiche della SISM per il 2011: nonostante la concomitanza con il MCM 2011 sono state, infatti, organizzate tre Scuole, che ci auguriamo riscuotano buon successo sia per l'attualità delle tematiche, sia per la loro significativa valenza formativa garantita, come di consueto, dalla particolare attenzione che i responsabili pongono nella scelta di docenti di elevato valore scientifico e professionale.

Nella parte più strettamente scientifica, troverete due articoli dei vincitori del Premio SISM 2010 e due di altri giovani Soci. Vi invito a leggerli: apprezzerete, oltre alla qualità scientifica, anche la cura con cui questi lavori sono stati preparati, indice di maturità professionale e di attenzione da parte degli Autori per la Società e per la rivista, che ne è organo ufficiale. Per tutti noi del Consiglio Direttivo è motivo di grande soddisfazione ricevere articoli di buon valore, che testimoniano tangibilmente l'ottimo livello della microscopia in Italia e l'eccellente formazione dei nostri giovani ricercatori.

La Società nel suo insieme, con il costante impegno dei Soci nella promozione dell'offerta formativa e nella salvaguardia della qualità scientifica, può essere giustamente orgogliosa di questo risultato.

E ora vi lascio alla lettura della rivista e vi invio un saluto caloroso, nella certezza di incontrarvi numerosissimi, in Settembre, a Urbino.

Manuela Malatesta

Consiglio direttivo della SISM

Verbale della riunione del 18 giugno 2010

*Istituto Superiore di Sanità, Dipartimento di Tecnologie e Salute,
Viale Regina Elena 299, Roma*

Il giorno 18 giugno 2010, alle ore 10:30, presso il Dipartimento di Tecnologie e Salute dell'Istituto Superiore di Sanità, Viale Regina Elena, 299 a Roma, è convocata una riunione del Consiglio Direttivo della SISM per discutere il seguente OdG:

1. Approvazione del verbale della riunione precedente
2. Approvazione bilancio 2009 e aggiornamento situazione economica della Società
3. Attività 2010: resoconti e stato organizzativo
4. Proposte attività 2011
5. Stato organizzativo MCM2011 e proposte IFSM
6. Rivista "Microscopie" e ratifica vincitori concorso Copertina
7. Sito web
8. Premio SISM
9. Approvazione ammissione nuovi soci
10. Varie ed eventuali

Sono presenti: *Roberto Balboni, Fabio Biscarini, Elisabetta Falcieri, Amelia Montone, Mario Raspanti e Andrea Tombesi.*

Assenti giustificati: *Manuela Malatesta.*

Presiede *Amelia Montone*; svolge le funzioni di segretario verbalizzante *Roberto Balboni.*

1. Il verbale della riunione del Direttivo del 8 febbraio 2010 viene approvato all'unanimità.
2. Il Presidente illustra il bilancio della Società per l'esercizio 2009. Il Consiglio approva all'unanimità. Il Presidente illustra inoltre la situazione economica alla data presente.
3. Il Workshop "Contributi delle microscopie allo sviluppo delle nanotecnologie in campo biomedico: nanodrug delivery", organizzato a Roma, presso l'Istituto Superiore di Sanità, ha avuto una buona partecipazione con 25 partecipanti esterni. Dal punto di vista economico, ha raggiunto un sostanziale pareggio.
La "Scuola introduttiva teorico-pratica di Microscopia a Scansione di Sonda", organizzata a Bologna dal Dott. Fabio Biscarini ha avuto 17 partecipanti. Sono stati presenti diversi sponsors specifici per la Scuola, che hanno quindi contribuito positivamente al bilancio economico della manifestazione. La Scuola teorico-pratica di "Microscopia Elettronica in Trasmissione nella Scienza dei Materiali" è prevista per il mese di Novembre 2010, con organizzazione a cura del Dott. Roberto Balboni e del Dott. Andrea Migliori. La scuola verrà spezzata in due settimane e la parte pratica è prevista per il mese di febbraio 2011. Hanno aderito FEI, JEOL, Assing e Bruker. Si raccomanda agli organizzatori di definire nel calendario gli spazi per la partecipazione delle ditte.

4. Il Dott. Biscarini avanza la proposta di organizzare una Scuola avanzata di microscopia a sonda. Resta ancora da decidere se dedicarla a materiali, alle applicazioni biomedicali o ad entrambe. La Dott.ssa Amelia Montone è disponibile ad organizzare una Scuola SEM, nella quale si potrebbe introdurre l'utilizzo di SEM remoto.
5. Si decide di proporre Marco Vittori quale delegato presso l'IFSM.
La Prof.ssa Elisabetta Falcieri relaziona circa lo stato organizzativo del Congresso MCM2011 di Urbino. Sono state decise le quote, comprensive di pasti e trasporti. È stata fatta una convenzione con l'azienda trasporti per 6 bus a disposizione durante tutte le giornate per il trasporto dei partecipanti da e per il centro di Urbino. È stato contattato il comune per la Sala del Collegio Raffaello. Occorre identificare chairmen e invited speakers per le sessioni plenarie. Si chiede al direttivo che vengano formulate proposte per gli invited speakers.
6. Si richiede ai responsabili del Workshop e della Scuola effettuate di preparare una relazione conclusiva per la pubblicazione sulla Rivista della Società.
Il Consiglio Direttivo ratifica i vincitori del concorso "Copertina" della Rivista. Risultano vincitori i soci Dott.ssa Annalisa Aurora (ENEA C.R. Casaccia, Roma) per le Scienze dei Materiali e il Dott. Alberto Foglia (Lugano-Paradiso, CH) per le Scienze Biomediche.
7. È stato formulato un preventivo di massima per la riorganizzazione del sito web da parte del Dott. Cosimo Elefante, che è l'attuale gestore. Il Dott. Tombesi illustra brevemente quali sono gli obiettivi dell'operazione e manderà una proposta di riorganizzazione ai membri del Direttivo.
8. Il Presidente comunica le graduatorie dei candidati al Premio SISM 2010, per i settori Biomedico e Scienza dei Materiali.
9. Il Consiglio Direttivo approva l'ammissione dei soci:
Dott.ssa Francolini Maura, Dott. Mantecca Paride, Dott.ssa Conti Sara, Dott. Cultrera Massimiliano, Dott. Fabbricatore Tiziano, Dott. Liscio Andrea, Dott.ssa Pellegrino Teresa, Dott.ssa Giagnacovo Marzia, Dott. Falqui Andrea, Sig. Puleri Federico, Dott. Ponzoni Andrea, Dott. Iurlo Matteo, Dott. Bonandini Luca, Dott. Alessandro Gambardella, Dott.ssa Eva Bystrenova, Dott. Francesco Valle, Dott.ssa Aessia Persico
10. Viene concesso il patrocinio al Corso di Microscopia Confocale che si terrà a Modena il 23-24 Settembre 2010.

Alle ore 13:45, null'altro essendovi da deliberare, il Presidente dichiara chiusa la seduta.

*Amelia Montone
Roberto Balboni
Fabio Biscarini
Elisabetta Falcieri
Mario Raspanti
Andrea Tombesi*

Consiglio direttivo della SISM

Verbale della riunione del 26 novembre 2010

*Istituto di Microelettronica e Microsistemi del CNR,
Via P. Gobetti 101, Bologna*

Il giorno 26 novembre 2010, alle ore 11:30, presso l'Istituto di Microelettronica e Microsistemi del CNR, via P. Gobetti 101, Bologna, è convocata una riunione del Consiglio Direttivo della SISM per discutere il seguente OdG:

1. Approvazione del verbale della riunione precedente
2. Situazione economica della Società
3. Resoconto attività SISM 2010
4. Discussione attività SISM 2011
5. Stato organizzativo MCM2011
6. Rivista "Microscopie"
7. Sito web
8. Approvazione ammissione nuovi soci
9. Varie ed eventuali

Sono presenti: *Roberto Balboni, Manuela Malatesta, Amelia Montone, Mario Raspanti e Andrea Tombesi.*

Assenti giustificati: *Elisabetta Falcieri, Fabio Biscarini.*

Presiede *Amelia Montone*; svolge le funzioni di segretario verbalizzante *Roberto Balboni.*

1. Il verbale della riunione del Direttivo del 18 giugno 2010 viene approvato all'unanimità.
2. Il Presidente illustra la situazione economica attuale della Società. La scuola di Bologna ha avuto una larga partecipazione riempiendo tutti i posti disponibili; si prevede pertanto che potrà fornire un buon contributo economico alla Società.
3. Delle precedenti Scuole 2010 si è già relazionato nel precedente Direttivo. La Scuola TEM in Scienza dei Materiali di Bologna è attualmente in corso e si concluderà nel mese di febbraio 2011.
4. Biscarini ha confermato la disponibilità ad organizzare una Scuola avanzata di microscopia a sonda. Resta ancora da decidere se eventualmente estendere la scuola alle applicazioni biomedicali. Tombesi ritiene che una Scuola di analisi di immagine per tutte le tecniche microscopiche possa risultare di interesse. Balboni, visto che sono pervenute molte richieste per la Scuola TEM di Bologna anche da parte di ricercatori del campo biomedico, propone di organizzare una Scuola TEM di livello base rivolta in modo specifico alle discipline biomedicali. Dopo una breve discussione si decide di proporre tale scuola a Modena (referente Andrea Tombesi), dove esiste sia la possibilità di usare il TEM che la disponibilità di aule per lezioni frontali

ed informatiche. La Scuola sarà organizzata su tre giorni, dei quali 1 per la parte teorica e 2 per quella pratica. Il periodo verrà deciso in base alla disponibilità delle aule e in modo da non interferire con le altre iniziative SISM, ed in particolare col MCM2011.

Montone dà la disponibilità per l'organizzazione di una scuola SEM teorico-pratica.

5. L'organizzazione del MCM2011 di Urbino sta procedendo speditamente: Il sito è stato aggiornato e sono presenti i Topics, concordati con i componenti dell'International Advisory Board e del Scientific Programme Committee, si sta ultimando la brochure, sono state individuate le plenary lectures, diverse compagnie hanno prenotato gli spazi espositivi, il Congresso ha ricevuto l' "EMS extension", l'Università di Urbino concede tutti gli spazi gratuitamente ed un finanziamento, si stanno organizzando tutti gli aspetti logistici.
6. Il Direttore Responsabile della Rivista "Microscopie", Manuela Malatesta, relaziona sul prossimo numero. Saranno inclusi l'Elenco delle attività della Società ed il resoconto sulla Scuola TEM. Verrà dato ampio spazio al congresso di Urbino, verrà pubblicata una pagina di presentazione dell'organizzatrice Elisabetta Falcieri e, oltre ai contenuti scientifici, verranno anche date informazioni sulla città e la regione circostante.
7. Tombesi riferisce sull'aggiornamento del software per i pagamenti tramite carta di credito sul sito web che è stato imposto dalla banca di appoggio e che ha creato diversi problemi. Ora la situazione dovrebbe essere ritornata alla normalità.
Il Direttivo approva il trasferimento del sito web su di un provider on-line nella forma attuale; delibera altresì di affidare al Dott. Cosimo Elefante, che già si occupa della manutenzione del sito, questa fase.
Tombesi comunicherà ai consiglieri una proposta di riorganizzazione delle pagine del sito.
8. Il Consiglio Direttivo approva l'ammissione dei soci: Ing. Cattaneo Laura, Ing. Sacco Elisabetta.
9. Nulla da deliberare.

Alle ore 13:45, null'altro essendovi da deliberare, il Presidente dichiara chiusa la seduta.

*Amelia Montone
Roberto Balboni
Manuela Malatesta
Mario Raspanti
Andrea Tombesi*

Bando
per l'assegnazione del
Premio "Carla Milanesi"

La SISM, in collaborazione con le Ditte del settore della Microscopia, bandisce un Premio riservato a giovani ricercatori non strutturati che siano Soci SISM o abbiano fatto domanda di associazione alla SISM entro il 5 marzo 2011, che presentino un contributo scientifico al MCM 2011 (10th Multinational Congress on Microscopy 2011: www.mcm2011urbino.it).

Ai due migliori contributi presentati personalmente, uno per il settore biomedico ed uno per scienza dei materiali, verrà assegnato il

PREMIO "Carla Milanesi"

dell'importo di € 500,00 ciascuno.

I partecipanti devono essere in regola con le quote associative ed inviare

- Copia dell'abstract inviato al Congresso

Per partecipare alla selezione del bando, che verrà effettuata a giudizio insindacabile del Consiglio Direttivo, occorre inviare la documentazione richiesta per e-mail al Presidente della SISM, Dott.ssa Amelia Montone (amelia.montone@enea.it).

I partecipanti in possesso dei requisiti necessari possono partecipare anche ai Contributi di partecipazione al MCM 2011.

La scadenza per l'invio della documentazione coincide con la data di scadenza dell'invio degli abstracts al MCM 2011.

Al ricevimento della documentazione verrà inviata una e-mail di conferma dell'avvenuta ricezione.

È fatto obbligo partecipare a tutta la durata del Convegno, pena esclusione dalla graduatoria.

I risultati della selezione verranno pubblicizzati sulla pagina web della SISM all'indirizzo: www.sism.it

Bando per l'assegnazione di
Contributi di partecipazione al
10th Multinational Congress on Microscopy

4 - 9 settembre 2011, Urbino

La Società Italiana Scienze Microscopiche (SISM), in collaborazione con le Ditte del settore della Microscopia, bandisce

n. 6 CONTRIBUTI

dell'importo di € 500,00 ciascuno per favorire la partecipazione di giovani ricercatori italiani al MCM 2011 (10th Multinational Congress on Microscopy 2011: www.mcm2011urbino.it) che si terrà ad Urbino in Italia dal 4 al 9 Settembre 2011.

I Contributi di partecipazione sono riservati a giovani ricercatori che non hanno una posizione permanente.

I partecipanti devono inviare:

- 1) Copia dell'abstract inviato al Congresso.
- 2) Un Curriculum Vitae di massimo due pagine con autocertificazione della propria posizione di "non strutturato".
- 3) L'iscrizione alla SISM, a parità di giudizio, costituirà titolo preferenziale.

Per partecipare alla selezione del bando, che verrà effettuata a giudizio insindacabile del Consiglio Direttivo, occorre inviare la documentazione richiesta per e-mail al Presidente della SISM, Dott.ssa Amelia Montone (amelia.montone@enea.it).

I partecipanti in possesso dei requisiti necessari possono partecipare anche al Premio "Carla Milanese".

La scadenza per l'invio della documentazione coincide con la data di scadenza dell'invio degli abstracts al MCM 2011.

Al ricevimento della documentazione verrà inviata una e-mail di conferma dell'avvenuta ricezione.

È fatto obbligo partecipare a tutta la durata del Convegno, pena esclusione dalla graduatoria.

I risultati della selezione verranno pubblicizzati sulla pagina web della SISM all'indirizzo: www.sism.it.

Elenco delle attività promosse dalla SISM nel 2011

La SISM organizzerà anche quest'anno diverse attività che, per l'importanza e la attualità degli argomenti trattati, la valenza scientifica dei relatori e la possibilità di attività pratiche con strumentazioni tecnologicamente avanzate, sono rivolte a ricercatori e a personale tecnico qualificato impegnato nei diversi settori della Microscopia. Per ulteriori informazioni e per accordi sulle modalità di partecipazione (interventi, strumentazione, ecc.) si prega di contattare i direttori responsabili.

1. Scuola teorico-pratica di microscopia elettronica a trasmissione ed elaborazione di immagini - Livello base

Modena, C.I.G.S. (Università degli Studi di Modena e Reggio Emilia), 15-17 giugno 2011

La scuola, organizzata dalla SISM in collaborazione con il Centro Interdipartimentale Grandi Strumenti, ha come obiettivo quello di fornire principi e tecniche di base per l'utilizzo del microscopio elettronico a trasmissione ed è rivolta a ricercatori, studenti e tecnici che sono interessati alla sua applicazione in ambito biomedico e dei materiali.

La scuola prevede una parte teorica sul TEM (principi di base sulla formazione dell'immagine, segnali, microanalisi), alcune lezioni sulle modalità e problematiche legate alla preparazione dei campioni e lezioni di base sui principi e tecniche di elaborazione delle immagini digitali.

La scuola sarà organizzata con lezioni teoriche e pratiche presso le aule e i laboratori del CIGS e comprende anche esercitazioni di analisi di immagini in un laboratorio di informatica.

Per informazioni: Dott. Andrea Tombesi (andrea.tombesi@unimore.it)

2. Scuola teorico-pratica di Microscopia Elettronica a Scansione in Scienza dei Materiali

Roma, ENEA C.R. Casaccia, 4-6 ottobre 2011

La scuola, organizzata dalla SISM in collaborazione con l'ENEA, tratterà i principi della microscopia elettronica a scansione e le sue applicazioni nel campo della Scienza dei Materiali ed è rivolta a ricercatori, studenti e tecnici interessati alla microscopia e a chi opera nel campo dei materiali. La scuola prevede una parte teorica sul SEM (elementi di ottica elettronica, interazione elettrone-materia, rivelatori e segnali, microanalisi); una parte pratica ai microscopi elettronici a scansione (sia a pressione variabile con filamento LaB₆ e microanalisi a raggi X sia FEG ad alta risoluzione) e presentazioni di novità strumentali. I microscopi sono corredati da sistemi di condivisione e controllo remoto per l'acquisizione delle immagini attraverso la rete web. Verrà illustrata la procedura di utilizzo delle apparecchiature in modalità remota da parte di utenti esterni.

È possibile osservare campioni portati dai partecipanti.

La scuola sarà organizzata con lezioni teoriche e pratiche presso i laboratori dell'ENEA C.R. Casaccia per la durata di tre giorni e di una sessione in remoto per l'osservazione dei campioni portati dai partecipanti attraverso la condivisione del microscopio via web.

È previsto un test di valutazione finale per gli studenti interessati a richiedere il riconoscimento di crediti formativi universitari (CFU).

Per informazioni: Dott.ssa Amelia Montone (amelia.montone@enea.it)

3. Scuola Avanzata di Microscopia a Scansione di Sonda

Bologna, CNR-ISMN, ISOF, 28 novembre - 3 dicembre 2011

La scuola Avanzata di Microscopia a Scansione di Sonda (Advanced Scanning Probe Microscopy - Advanced SPM) si prefigge lo scopo di insegnare gli aspetti teorici e pratici delle tecniche a scansione di sonda più specifiche. Esse vengono utilizzate per studiare le proprietà chimico/fisiche superficiali quali, ad esempio, la distribuzione della carica elettrica (Phase-EFM - Microscopia a Forza Elettrica misurata mediante la fase), la distribuzione del potenziale elettrico (KPFM - Microscopia di Forza a Sonda Kelvin) e la risposta meccanica (Fase della Microscopia a Forza Atomica, Microscopia a Forza Laterale, Microscopia a Forza Atomica Bimodale).

La scuola può essere considerata adatta a tutte le persone che hanno conoscenza della microscopia SPM e sono interessati ad approfondirla o ad espandere le capacità d'uso del proprio microscopio. La scuola avrà la durata di 5 giorni. I primi due giorni saranno dedicati alla teoria, mentre i restanti giorni alla parte sperimentale.

Per informazioni: Dott. Fabio Biscarini (f.biscarini@bo.ismn.cnr.it), Dott. Cristiano Albonetti (c.albonetti@bo.ismn.cnr.it)

Resoconto della scuola SISM**Scuola teorico-pratica di Microscopia Elettronica
in Trasmissione in Scienza dei Materiali**

22-26 novembre 2010; 7-11 febbraio 2011

Bologna, Istituto CNR - IMM

Responsabili: Roberto Balboni e Andrea Migliori

Con la realizzazione delle sessioni pratiche al microscopio dal 1 al 11 Febbraio 2011, si è conclusa la Scuola di Microscopia Elettronica in Trasmissione in Scienza dei Materiali 2010. Le lezioni teoriche si erano svolte dal 22 al 26 Novembre scorso, ricalcando un format (due settimane di corso fra loro separate) già sperimentato nel 2008 e che ha riscosso il favore di tutti i partecipanti. A partire da questa edizione la scuola è intitolata a Pier Giorgio Merli, che ne è stato l'ideatore nel 2005. L'evento ha rappresentato uno dei momenti dell'attività SISM 2010 ed ha quindi visto la partecipazione di ditte quali FEI che ha effettuato una presentazione sulla preparazione di campioni mediante FIB e di Assing e Bruker che hanno fornito materiale informativo ai partecipanti.

Gli argomenti trattati hanno spaziato dalla strumentazione, all'interazione elettrone-materia, alla teoria base e cinematica/dinamica della diffrazione elettronica, fino alle tecniche CBED, HREM, STEM, EDX e EELS. Oltre 30 ore di lezioni in aula, e 25 ore di sessioni pratiche allo strumento hanno costituito un impegno importante, sia per gli allievi che per i docenti. Ma gli obiettivi che ci eravamo posti erano ambiziosi e richiedevano impegno. In primo luogo descrivere con completezza i principi di funzionamento dello strumento e delle tecniche di indagine, in un periodo in cui le sempre più sofisticate interfacce software di utilizzo, a fronte di una maggiore facilità d'uso e miglioramento della produttività, spesso non richiedono una loro conoscenza approfondita. In secondo luogo dare la possibilità ai partecipanti di operare realmente al microscopio, nelle diverse tecniche trattate.

Ce l'abbiamo fatta? La scuola ha visto 9 partecipanti alle sessioni pratiche (su 8 posti previsti) e 11 alle lezioni teoriche, provenienti da università e istituti di ricerca distribuiti su tutto il territorio nazionale: Università e Politecnico di Torino, Politecnico di Milano, Università di Modena, Istituti CNR di Faenza e Messina e Laboratori INFN di Frascati. Questo è stato senz'altro un primo successo, in un momento in cui la partecipazione ad un evento così impegnativo può non essere alla portata di tutti i laboratori. Completate le lezioni, sono comunque gli studenti che hanno avuto il compito di comporre il giudizio finale della scuola: a questo scopo è stato distribuito un questionario alla fine delle lezioni e sono stati dedicati spazi alla discussione comune sull'andamento della Scuola sia alla fine delle lezioni teoriche che delle sessioni pratiche. I risultati sono stati senz'altro lusinghieri, constatando che la media dei voti delle lezioni si aggira attorno a 8,5 mentre la valutazione complessiva della Scuola arriva quasi a 9. E dai giudizi espressi emerge la soddisfazione di aver anche potuto apprendere e approfondire argomenti poco conosciuti o, in alcuni casi, ignorati. Non sono comunque mancate le osservazioni critiche rispetto ad alcuni contenuti, risultati di difficile comprensione, e soprattutto rispetto alla generale difficoltà ad assimilare una grande mole di nozioni in poco tempo. Nelle sessioni pratiche poi l'interattività è diventata più immediata, ed è stato più facile per gli allievi porre domande, operare e verificare teorie. Emblematico, durante la discussione finale a conclusione della parte pratica, ovvero dopo una settimana di impegnative sessioni al microscopio e di simulazione, assistere ad allievi che chiedevano la possibilità di un'ulteriore settimana di pratica su argomenti specifici a docenti dal viso attonito e dall'occhio spento...!

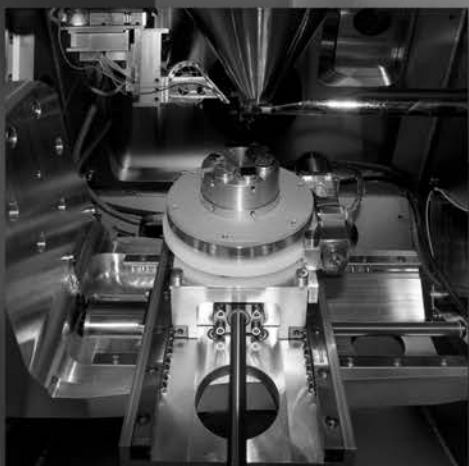
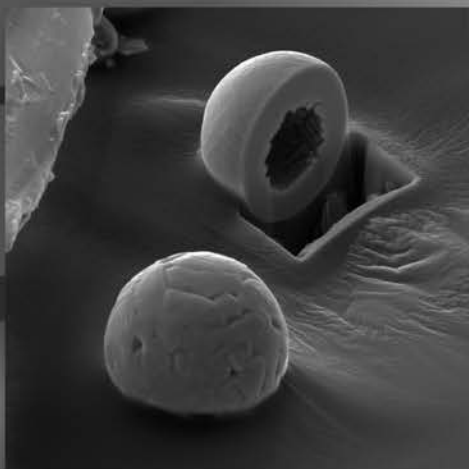
In conclusione, pensiamo di poter affermare che l'edizione 2010 della Scuola TEM in Scienza dei Materiali abbia rappresentato un'occasione senz'altro positiva per tutti. Al di là dei meri aspetti didattici, due settimane di interazione fra ricercatori interessati alla microscopia elettronica hanno creato una rete di relazioni, informazioni e scambio che hanno sicuramente rappresentato un valore aggiunto per allievi e docenti.

Un'esperienza da ripetere quindi? Noi ci contiamo proprio: arrivederci al 2012!

*Roberto Balboni
Andrea Migliori*

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10th Multinational Congress on Microscopy 2011

4-9 Settembre 2011, Urbino

Cari Amici, Colleghi, Soci,

Vi scrivo per darvi ulteriori dettagli sul MCM 2011, da tempo in corso di organizzazione. Come sapete dal precedente annuncio, il congresso si svolgerà interamente presso il Campus Scientifico dell'Università, che sarà quasi completamente a nostra disposizione, essendo in un periodo di assenza di didattica frontale.

Il programma scientifico non è ancora definitivo, ma numerosi autorevoli ricercatori internazionali hanno accettato il nostro invito a presentare contributi nelle varie sessioni, che saranno moderate da altrettanti noti esperti nelle specifiche materie. Nel sito www.mcm2011urbino.it, in continuo aggiornamento, troverete tutte le informazioni.

Le sessioni plenarie vedranno la presenza di G. Van Tendeloo (Antwerp, Belgio), A. Kovacs (Copenhagen, Danimarca), V. Radmilovic (Berkley, USA), M. Schreiner (Vienna, Austria), A. Nanci (Montreal, Canada), S. Cinti (Ancona, Italia) e J. Carrascosa (Madrid, Spagna), tutti ricercatori di altissimo livello nel campo delle Strumentazioni e Metodologie, nella Scienza dei Materiali o nelle Scienze della Vita, i tre ambiti che, da sempre, caratterizzano i Multinational Congress on Microscopy.

Le sessioni specifiche, organizzate dall'International Advisory Board e dal Scientific Programme Committee, ospiteranno relatori ad invito provenienti dai paesi che tradizionalmente costituiscono l'anima del Multinational Congress (Austria, Croazia, Repubbliche Ceca e Slovacca, Ungheria, Serbia, Slovenia e Italia), oltre a numerosi autorevoli ricercatori di altri paesi europei. Saranno benvenuti altri contributi sia in forma di presentazione orale che come poster, che verranno valutati dal Scientific Programme Committee e attribuiti alle varie sessioni, nella forma opportuna e nel giorno dedicato.

Le sessioni plenarie si svolgeranno nell'Aula Magna del Campus Scientifico, aula da 300 posti, attrezzata con microfoni individuali e con salette limitrofe di supporto, mentre le sessioni a tema saranno nelle altre aule del Campus, con numero di posti variabile da 20 a 80.

Le sessioni poster si svolgeranno in altri spazi dedicati, in prossimità dell'Aula Magna e della mostra strumentale.

Nel sito sono presenti le scadenze relative all'iscrizione: come nei precedenti congressi, le quote di iscrizione variano a seconda della categoria dei partecipanti (membri o non membri della European Microscopy Society, dipendenti delle compagnie espositrici) e in base al momento dell'iscrizione (fino al 30 aprile, dal 1 maggio al 30 giugno, dal 1 luglio fino ai giorni del congresso).

Come potete vedere nel sito, siamo riusciti a tenere le quote di partecipazione molto basse, in particolare per i giovani non strutturati, alla cui partecipazione teniamo particolarmente. Per ogni categoria di iscritti, la registrazione comprende, oltre all'accesso a tutte le strutture del congresso dalla domenica pomeriggio al venerdì, il libro dei Proceedings con CD-Rom, tutti i pranzi e i coffee breaks, il party di benvenuto, il farewell party e tutti i trasferimenti tra campus, residenze universitarie, hotel e centro di Urbino.

Avendo il MCM 2011 avuto l'extension da parte della European Microscopy Society, potrà usufruire, oltre che di un supporto economico per sostenere qualche speaker internazionale, di un certo numero di borse di partecipazione per giovani, offerte dall'EMS, secondo criteri che verranno comunicati tramite il sito.

La SISIM, inoltre, come da documentazione trasmessa a tutti i soci dalla Presidente Amelia Montone, per favorire la partecipazione dei giovani al congresso, ha emesso un bando per l'assegnazione di "6 contributi di partecipazione" e di "2 premi Carla Milanese", in entrambi i casi di 500 euro. Nel sito SISIM troverete tutte le informazioni al riguardo.

In linea con quanto fatto al congresso di Graz 2009, verranno anche organizzati dei "premi poster", sia nell'ambito della strumentazione, che in quello della scienza di materiali, che nelle scienze della vita.

Gli ospiti potranno utilizzare le Residenze Universitarie (fino a 300 posti), con varie tipologie di stanze

e con tariffe giornaliere da 17 a 30 euro a notte. Le stanze, durante l'anno accademico in uso agli studenti e per l'occasione a disposizione del Congresso, sono collocate presso il Campus Scientifico, nel Collegio del Colle e nel Collegio Internazionale; un servizio autobus a carico dell'organizzazione garantirà tutti i trasporti.

Gli Hotel (fino a 200 posti) - S. Domenico, Italia, Nuovo, Bonconte, Dei Duchi, Mamiani, Raffaello, Tortorina, Piero della Francesca - tutti collocati in centro città o nelle strette vicinanze, hanno dato la disponibilità a prezzi in convenzione con l'Università, a partire da 35 euro a notte, che si possono reperire nel sito. Come per le Residenze Universitarie, un servizio autobus dedicato garantirà tutti i trasporti.

L'esposizione commerciale avrà luogo in appositi spazi del Campus in prossimità dell'Aula Magna. Nello spazio al piano terra, più consono alla collocazione di strumenti, esporranno, in box opportunamente attrezzati, Agilent, Assing, Bruker, Cameca, Diatome, FEI, Gatan, Jeol, Leica, Nikon, Oxford Instruments, Quorum, SPI Supplies, Technoorg, Tescan e altre, con trattativa ancora in corso. In uno spazio al secondo piano si prevedono stand di case editrici e/o di espositori locali. In una parte della mostra espositiva, oltreché vicino alla reception, verrà allestita un'area computer, anche se tutti gli spazi del Campus possono usufruire di sistema wireless.

Il Congresso sarà preceduto da un Meeting Satellite su "Tomography, 3D Reconstruction and 3D Imaging Techniques", organizzato ad Ancona da Paolo Mengucci sabato 3 settembre pomeriggio e domenica 4 settembre mattina. Il meeting, che si svolgerà presso la sede del Rettorato di Ancona, vedrà relatori in parte presenti anche ad Urbino, altri autorevoli esperti nel campo specifico e tecnici delle compagnie più avanzate nel settore della tomografia. Tutte le informazioni verranno progressivamente aggiornate sul sito del MCM 2011.

La cena sociale sarà giovedì 8 settembre presso la Sala Raffaello, una sala storica circondata da un doppio chiostro, all'interno di un prestigioso palazzo rinascimentale del centro di Urbino.

Il programma sociale per gli accompagnatori comprenderà visite guidate a Palazzo Ducale, agli Oratori delle Confraternite, alla Casa di Raffaello, oltre ad escursioni ai siti di interesse storico-artistico della regione e, a richiesta, al mare.

Nella speranza di offrire contenuti di alto livello scientifico e tecnologico, oltre a giornate di proficua e serena convivialità in un soleggiato settembre, vi aspettiamo numerosi ad Urbino. A presto,

Elisabetta Falcieri
Presidente del MCM2011

elisabetta.falcieri@uniurb.it
elisabetta.falcieri@mcm2011urbino.it
info@italymeeting.it
www.mcm2011urbino.it

URBINO MCM 2011



September 4 - 9, 2011

Scientific Campus, Urbino University "Carlo Bo", Italy



10th Multinational Congress on Microscopy 2011

Italian Society of Microscopical Sciences

Austrian Society for Electron Microscopy

Croatian Microscopy Society

Czechoslovak Microscopy Society

Hungarian Society of Microscopical Sciences

Serbian Society for Microscopy

Slovene Society for Microscopy

www.mcm2011urbino.it

September 4 - 9, 2011
 Scientific Campus, Urbino University "Carlo Bo", Italy

URBINO MCM 2011



Dear Colleagues,

It is with great pleasure that we announce the 10th Multinational Congress on Microscopy, which will be organized in Italy on September 4-9 2011. MCM 2011 will join proposals from the Austrian Society for Electron Microscopy, Croatian Microscopy Society, Czechoslovak Microscopy Society, Hungarian Society of Microscopical Sciences, Serbian Society for Microscopy, Slovene Society for Microscopy and Italian Society of Microscopical Sciences.

The congress venue will be Urbino, a middle - age city in the centre of Italy, with a numerous and ancient university, a pleasant climate and a beautiful nature.

Both scientific program and trade exhibition are in progress in the University Campus, a wide modern building, equipped with a main hall, eight 20-80 seat lecture rooms, a bar/cafeteria and a large space for commercial stands.

The Campus is located on a hilly floor and offers a number of terraces and grassy areas, where coffee breaks, lunches and, eventually, dinners will be organized. 100 University Residence rooms, located close to exhibitor spaces and to scientific session areas, are available for delegates and exhibitors. In addition, two more University Residences and a number of low price hotels can be offered in Urbino historical centre.

As in the previous multinational congresses, the three main scientific areas - Instrumentation and Methodology, Materials Science and Life Sciences - will represent the framework of the congress.

The program comprises plenary lectures, keynote lectures, poster sessions, symposia and workshops.

During the whole congress a high level Trade Exhibition, displaying modern instruments and devices, addressed to all fields of microscopy in physical and life sciences, will be organized in two large spaces located close to the lecture halls and facing the garden or terrace spaces.

As suggested in Graz Congress, we are trying to keep conference fees low and all including. In particular, the participation of students, also encouraged by the very convenient prices of University Residence rooms, is friendly welcome. Fellowships and poster prizes will also further stimulate young people scientific contributions.

The registration before April 30, 2011 is particularly convenient both for EMS members/non members and for students.

The organization of a Satellite Meeting on Tomography and 3D Reconstruction is in progress, on September 3 and 4 morning, in Ancona, a lovely sea-side University City about 100 Km far from Urbino, and the transfer to the congress venue will be planned by the organization. This event will represent a further occasion to investigate a promising field of the microscopy that concerns both life and material science. It will also be a helpful opportunity for young people to discuss problems and exchange opinions with expert scientists.

The workshop will take place at the Rectorate building of the Marche Polytechnique University located at the town centre. Low fees and university residence rooms offered at very low prices will be available to encourage the participation of students and young people. For further information on this event, please, contact the local organizers at: p.mengucci@univpm.it.

Finally, we invite you to participate numerous and make MCM2011, with your contributions, a stimulating occasion of scientific and technological exchanges, as well a moment of pleasant meeting in an interesting city full of art and history, surrounded by fascinating green hills.

Sincerely

Elisabetta Falcieri,

president of MCM2011 Urbino

elisabetta.falcieri@uniurb.it; elisabetta.falcieri@mcm2011urbino.it

www.mcm2011urbino.it

www.mcm2011urbino.it

September 4 - 9, 2011
 Scientific Campus, Urbino University "Carlo Bo", Italy

URBINO MCM 2011

Italian Society of Microscopical Sciences
 Austrian Society for Electron Microscopy
 Croatian Microscopy Society
 Czechoslovak Microscopy Society
 Hungarian Society of Microscopical Sciences
 Serbian Society for Microscopy
 Slovene Society for Microscopy

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www.mcm2011urbino.it

September 4 - 9, 2011
Scientific Campus, Urbino University "Carlo Bo", Italy

URBINO **MCM** 2011

Congress Topics

Instrumentation and Methodology

- I1 Advanced techniques for atomic resolution
- I2 High resolution TEM and STEM
- I3 Electron diffraction techniques and analytical TEM
- I4 Advances in SEM and HR-SEM
- I5 Advances in optical and scanning probe microscopy
- I6 3D-Imaging and nanotomography
- I7 Advances in sample preparation techniques
- I8 Environmental microscopy, X-ray and ion microscopy

Materials Science

- M1 Materials for nanotechnology
- M2 Carbon based materials
- M3 Semiconductors and magnetic materials
- M4 Thin films, coatings, surfaces and interfaces
- M5 Metal alloys and intermetallics
- M6 Ceramics, composites, minerals and self-assembled materials
- M7 Polymers and soft materials
- M8 Microscopy in cultural heritage

Life Sciences

- L1 Multidisciplinary approaches in biology and medicine
- L2 High resolution light microscopy in biological sciences
- L3 Live cell imaging
- L4 Ultrastructural pathology
- L5 Microscopical applications in environmental science
- L6 Extracellular matrix and calcified tissues
- L7 Structure and Imaging of biomolecules
- L8 3D subcellular architecture by electron tomography



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URBINO MCM 2011

Information

Urbino is a middle-age city in the centre of Italy, with a numerous and ancient University, a pleasant climate and a beautiful nature.

Location map



HOW TO REACH URBINO

By airplane: To Rimini (79 Km from Urbino), Ancona-Falconara (90 Km), Forli (133 Km) or Bologna (173 Km) airports. Take the train to Fano or Pesaro and continue by bus (<http://www.adriabus.eu/>)

By ship: To Pesaro (36 Km from Urbino), Fano (90 Km), Cesenatico (95 Km), Rimini (79 Km) or Ancona (90 Km) ports. Take the train to Fano or Pesaro and continue by bus (<http://www.adriabus.eu/>).

By car: From Austria and Eastern Europe: through Tarvisio, take SS13, then highway A23-E55. Continue along highway E55. Take A57, A4, then A13 and A14. Take the exit PESARO-URBINO and continue along SP423.

By train: From the train stations of Fano or Pesaro there are buses going directly to Urbino, see above.

Congress Venue

The University Scientific Campus Sogesta,
 Via Cà Le Suore, 2 - 61029 Urbino PU.

Accommodation

University Residences: Scientific Campus,
 Collegio del Colle, Collegio Internazionale.

Hotels: S. Domenico, Italia, Bonconte, Mamiani,
 Dei Duchi, Piero della Francesca

Important Dates

ABSTRACT SUBMISSION AND
 REGISTRATION OPEN

January 30, 2011

ABSTRACT SUBMISSION DEADLINE

April 30, 2011

EARLY REGISTRATION DEADLINE

April 30, 2011

HOTEL RESERVATION DEADLINE

June 30, 2011

Conference Language

English

Contact Address

President of MCM2011

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 Sciences, Urbino, Italy

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LA MUTA - RAFFAELLO (GALLERIA NAZIONALE DELLE MARCHE)

URBINO MCM 2011







10th Multinational Congress on Microscopy 2011
September 4 – 9 2011
Scientific Campus Urbino University “Carlo Bo”, Italy

**Satellite workshop on
Tomography, 3D Reconstruction and 3D Imaging Techniques**
Ancona, 3-4 September 2011

Workshop venue

The workshop venue is the Palazzo del Rettorato located in Piazza Roma 22, Ancona.

The workshop venue is at the centre of Ancona at a walking distance from the ferry terminal, the railway station of Ancona Marittima and the university residence.

Programme

The workshop will begin on Saturday afternoon and will continue on Sunday morning.

At the end of the workshop participants will be transferred by bus to the congress venue at Urbino.

Accommodation

University residence rooms located at a block of flats about 400 meters far from the congress venue will be available free of charge, for a maximum of 22 persons. Accommodation is provided in double rooms inside a flat. Each flat is composed of a kitchen, two double rooms and a shared bathroom. Cleaning services, towels and bed-clothes included.

Alternatively, hotels of different categories are available at a walking distance from the workshop venue or nearby Ancona.

Moreover, a wide offer of hotels and any other kind of accommodation are available in the touristic towns located few kilometres from Ancona.



10th Multinational Congress on Microscopy 2011
 September 4 – 9 2011
Scientific Campus Urbino University “Carlo Bo”, Italy

Travel information

Plane: Ancona-Falconara “Raffaello Sanzio” airport 16 km, Rimini “Federico Fellini” airport 100 km, Bologna “Guglielmo Marconi” airport 220 km, Roma-Fiumicino “Leonardo da Vinci” airport 340 km

Train: Ancona Centrale 3 km, Ancona Marittima 700 m

Car: Motorway A14, Ancona nord 18 km, Ancona sud 12 km

Ferry: Ancona ferry terminal 700 m

Tourist information

www.turismo.marche.it

Fees

Members of participating societies and EMS members	€ 100,00
Non-members	€ 120,00
Students	€ 50,00

Contributi di partecipazione EMS al 10th Multinational Congress on Microscopy

Dear EMS member,

During the previous EMS Board meeting it was decided to select The Microscopy Conference MC 2011 (Kiel, Germany, August 28 - September 2, 2011) and the 10th Multinational Congress on Microscopy (MCM 2011, Urbino, Italy, September 4-9, 2011) as EMS Extensions for 2011.

To support young researchers several *scholarships of each 250 Euro* will be awarded by EMS for participation at one of these meetings (exact number to be decided). Application deadline for these scholarships equals the abstract submission deadline for each meeting (March 31 for MC 2011, Kiel and *April 30 for MCM 2011, Urbino*). Each applicant must submit at least one abstract and be registered at the meeting. Copy of abstract(s), registration details and proof of student or early stage researcher when applicable have to be added to the application. The application including a short CV should be sent to the EMS Secretary.

We look forward to your applications,

Nick Schryvers
EMS Secretary

Eventi nazionali

2011

Scuola di microscopia elettronica e spettrometria a raggi X applicate alla diagnostica dei beni culturali

Centro Conservazione e Restauro la Venaria Reale

11-13 maggio 2011

Venaria (Torino)

http://www.centrorestaurovenaria.it/pagine.cfm?LANG=IT&PAG_ID=347&SEZ_ID=22&TS_ID=20

XXXIV Congresso Nazionale della Società Italiana di Istochimica

7-9 giugno 2011

Pala Riviera, San Benedetto del Tronto

<http://www.istochimica2011.unicam.it>

XXIX CONFERENZA NAZIONALE DI CITOMETRIA

La Cellula. Ricerca Biologica, Diagnostica e Nuove Terapie. Le sfide della citometria

5-8 ottobre 2011

<http://biotec.casaccia.enea.it/GIC/>

Eventi internazionali

2011

EMAT Winter Workshop on Transmission Electron Microscopy 2011

January 17-28, 2011

University of Antwerp, Belgium

Organization: EMAT research group

BioImaging: Illumination of Disease

January 27-28, 2011

Queen's University Belfast, Northern Ireland, UK

Organization: RMS

AMAS XI

The eleventh biennial meeting of the Australian Microbeam Analysis Society (AMAS)

February 6-11, 2011

Canberra, Australia

Organization: the Australian Microbeam Analysis Society (AMAS)

FRET-FLIM workshop

February 8, 2011

Lambert Instruments headquarters in Roden, the Netherlands

Organization: Lambert Instruments, Nikon Instruments Europe

European Symposium: Current Developments in TEM

March 10-11, 2011

Freiberg University, Germany

Organization: Bergakademie Freiberg, JEOL

DPG Spring Meeting

Symposium: "New Developments in Transmission Electron Microscopy of Materials"

March 13-18, 2011

Dresden, Germany

Organization: German Physical Society (DPG)

Stereology Week 2011

March 22-25, 2011

Bordeaux, France

Organization: Explora Nova

Spring-Meeting of the PANOS working group on "Correlative Microscopy"

March 25, 2011

MPI Dortmund, Germany

Organization: MPI, MPG, DGE

EBSD 2011

March 28-29, 2011

Dusseldorf, Germany

Organization: RMS

EMBO Workshop: Chromatin structure, organization and dynamics

April 09-13, 2011

Prague, Czech Republic

Organization: EMBO

TEM and TOM Meeting

Aberration Corrected & Quantitative (S)TEM & Advances in Tomography III

April 11-12, 2011

University of Sheffield, UK

Organization: RMS

Microscopy of Oxidation 8

April 11-13, 2011

University of Liverpool, UK

Call for papers deadline: 30 September 2010

ImagineNano

April 11-14, 2011

Bilbao, Spain

Organizers: Fundación Phantoms, Donostia International Physics Center (DIPC), CIC nanoGUNE, País Vasco University, Bilbao Exhibition Centre (BEC)

Botanical Microscopy 2011

April 16-21, 2011

University of Wageningen, The Netherlands

Organization: RMS

Focus on Microscopy (FOM2011)

April 17-20, 2011

Konstanz University, Konstanz, Germany

The SOLEIL Synchrotron School on X-ray Microscopy (SOLEMIO)

May 2-6, 2011

Synchrotron SOLEIL, Saint-Aubin, France

Organization: Synchrotron SOLEIL

Sixth International Congress on Electron Tomography

May 5-8, 2011

EMBL Heidelberg, Germany

Organization: EMBL

43 Erice crystallographic course: "Electron crystallography"

EMS sponsored event

June 2-12, 2011

Ettore Majorana Centre, Erice, Italy

11th annual meeting of the European Light Microscopy Initiative (ELMI)

June 7-10, 2011

Hotel & Conference Center Thraki Palace, Alexandroupolis, Greece

Immunohistochemistry for GMA Resin Embedded Tissues

June 13-17, 2011

Southampton University Hospital, UK

Organization: Histochemistry Research Unit

13th Euroseminar on Microscopy Applied to Building Materials (EMABM 2011)

June 14-18, 2011

Ljubljana, Slovenia

The XIVth International Conference on Electron Microscopy

EMS sponsored event

June 26-30, 2011

Wisla, Poland

Organization: Institute of Materials Science University of Silesia, Poland, PTMi

Inter/Micro: 62nd Annual Applied Microscopy Conference

July 11-15, 2011

McCrone Research Institute, Chicago, IL, USA

Organization: McCrone Research Institute

Microscopy & Microanalysis 2011

August 7-11, 2011

Nashville, Tennessee, USA

Organization: MSA

Microscopy Conference MC 2011

EMS extension

August 28 - September 2, 2011

Kiel, Germany

Organization: DGE, SCANDEM, PTMi

10th Multinational Congress on Microscopy (MCM 2011)

EMS extension

September 4-9, 2011

Urbino, Italy

Organization: ASEM, CSEM, CSMS, HSM, SISM, SSM, SDM

EMAG 2011

September 6-9, 2011

University of Birmingham, UK

Organization: The Electron Microscopy and Analysis Group's (EMAG)

The EMBO Meeting 2011

September 10-13, 2011

Vienna, Austria

Organization: EMBO

20th National Electron Microscopy Congress

October 25-28, 2011

Antalya, Turkey

Organization: Turkish Society for Electron Microscopy

2012**Inter/Micro: 63rd Annual Applied Microscopy Conference**

July 9-13, 2012

Chicago, IL, USA

15th European Microscopy Congress, EMC 2012

September 16-21, 2012

Manchester Central Convention Complex, Manchester, UK

Organization: EMS, RMS, EMAG

Centro Grandi Strumenti

Università degli Studi di Pavia, Cascina Cravino, Via Bassi, 21 - 27100 Pavia, Italy

Presidente: Prof. Luigi Fabbrizzi

Direttore tecnico: Dott.ssa Federica Corana

E-mail: info@cgs.unipv.it - <http://cgs.unipv.it>



Centro Grandi Strumenti dell'Università degli Studi di Pavia, presso la Cascina Cravino.

Il Centro Grandi Strumenti (CGS) è un organismo interfacoltà creato allo scopo di acquisire e di gestire apparecchiature di particolare rilievo, di carattere il più possibile multidisciplinare, altrimenti non accessibili alle singole unità di ricerca dell'Ateneo Pavese. Il CGS si configura come centro di servizio interdipartimentale, ed è stato fondato nel 1984. Dal 1993 tutti i laboratori sono riuniti presso la sede del Centro in Cascina Cravino. Oltre ai laboratori sono disponibili presso il Centro: servizi grafici e informatici, laboratorio per la preparazione dei campioni, officina. Le *facilities* che il Centro mette a disposizione permettono a molti ricercatori dell'Università lo svolgimento di ricerche a livello di eccellenza internazionale. È tuttavia da notare che il Centro non rappresenta un semplice contenitore di sofisticate apparecchiature ma, nei vari Laboratori, sviluppa e approfondisce innovative ed avanzate metodologie di indagine.

Presso le strutture del Centro vengono svolti corsi e seminari specifici sullo sviluppo delle tecniche e metodologie impiegate, destinati a ricercatori e studenti. I Laboratori del Centro ospitano studenti di Facoltà dell'Ateneo Pavese, che ivi preparano le

tesi di laurea, e ricercatori provenienti da industrie di importanza nazionale, interessati all'acquisizione o all'approfondimento di tecniche di indagine di interesse applicativo.

Il Centro si articola in diversi Laboratori, ciascuno dedicato ad una complessa e sofisticata tecnologia di indagine, di interesse per i ricercatori dell'Università di Pavia: 1. Laboratorio di Microscopia Confocale; 2. Laboratorio di Cristallografia; 3. Laboratorio di Microscopia Elettronica; 4. Laboratorio di Risonanze Magnetiche; 5. Laboratorio di Spettrometria di Massa; 6. Laboratorio di Spettroscopie; 7. Laboratorio per la Struttura primaria delle proteine. La necessità di rinnovare la strumentazione in dotazione al Centro per assicurare che i gruppi di ricerca dell'Ateneo Pavese possano usufruire di tecnologie adeguate, si scontra con la recente riduzione dei fondi per il finanziamento delle Università. È dunque essenziale ricorrere a finanziamenti provenienti da Enti esterni. Il laboratorio di Microscopia Confocale del CGS nell'anno 2010 ha presentato una richiesta alla Fondazione Banca del Monte di Lombardia per ottenere un finanziamento per l'acquisto di un nuovo microscopio

confocale in sostituzione del microscopio *Leica TCS SP2* acquisito nel 2000. Il microscopio *Leica TCS SP2* è stato largamente utilizzato dai ricercatori pavesi nel decennio 2000-2010 ed è uscito di produzione nel 2007. Uno dei criteri fondamentali nella valutazione delle richieste sottoposte alla Fondazione risulta essere *il numero dei beneficiari del progetto*. Il Centro Grandi Strumenti essendo un centro di servizio interdipartimentale ha presentato una richiesta di finanziamento corredata dall'elenco e da un breve riassunto dei numerosi progetti di ricerca in cui è stato coinvolto il Laboratorio di Microscopia Confocale negli ultimi dieci anni (titoli dei progetti, fonti di finanziamento, responsabili dei gruppi di ricerca coinvolti). La larga e documentata condivisione dello strumento ha portato allo stanziamento da parte della Fondazione di una cospicua somma che, insieme a quanto messo a bilancio dal Centro, ha permesso di acquisire il nuovo microscopio confocale *LEICA TCS SP5 II*. Tale strumento soddisfa la gran parte delle esigenze degli utenti del Laboratorio di Microscopia Confocale, anche se sarà necessario nei prossimi mesi reperire i fondi per completare l'acquisto di alcuni software e accessori che lo renderebbero al massimo delle prestazioni. Il microscopio confocale *Leica TCS SP2* rimane a disposizione degli utenti e verrà mantenuto in attività compatibilmente con la disponibilità di fondi per coprire i costi di manutenzione straordinaria, e con la reperibilità delle parti di ricambio.

Laboratorio di Microscopia Confocale

Dott.ssa Patrizia Vaghi (Tel. 0382.987548)

Il Laboratorio di Microscopia Confocale è dotato di due microscopi confocali, un *Leica TCS SP2* e un *Leica TCS SP5 II*. Il microscopio confocale *Leica TCS SP5 II* è così articolato: 1) microscopio *Leica DMI 6000 CS* rovesciato, obiettivi in dotazione: 63x (NA 1.4), 40x (NA 1.25) e 20x (NA 0.7) tutti ad immersione in olio in modo da poter passare da uno all'altro senza spostare il vetrino o la petri col preparato; tutti gli obiettivi sono dotati di ottiche per UV; 2) lampade per luce trasmessa e per fluorescenza, kit per contrasto interferenziale; 3) sistema per microscopia confocale equipaggiato con le seguenti sorgenti di eccitazione: laser a diodi 405 con linea di emissione a 405 nm e laser RGB dicroico composto da un laser Ar/Vis con

linee di emissione a 458, 476, 488, 496 e 514 nm e da un laser HeNe con linee di emissione a 543 e 633 nm; 4) modalità di scansione: 2D (XY-XZ-ZT); 3D (XYZ-XZY-XYT-XZT-XYlambda-XZlambda); 4D 4(XYZT); 5) *software* *Leica* per l'acquisizione delle immagini in formato variabile fino a 8096x8096 pixel, dotato di più funzioni quali: ottimizzazione dell'immagine (contrasto, luminosità, filtri), stereo, rotazioni, volume rendering, simulazione 3D; 6) acquisizione simultanea su 3 canali 7) cameretta termostata per la programmazione e il mantenimento costante della temperatura; consente esperimenti *in vivo* anche di lunga durata.

La microscopia confocale nasce dall'esigenza di studiare tridimensionalmente preparati sia *in vivo* che sottoposti a fissazione senza sottoporli a tecniche distruttive quali tagli al criotomo o al criostato. I preparati per microscopia confocale vengono sottoposti a marcatura fluorescente o immunofluorescente ma l'innovazione di questa tecnica rispetto alla microscopia ottica convenzionale è costituita dal sistema ottico che consente il passaggio dell'emissione di fluorescenza emergente esclusivamente dai singoli piani focali dal preparato eliminando completamente l'interferenza dei piani fuori fuoco. Il preparato viene idealmente suddiviso in una griglia di punti che sequenzialmente vengono illuminati da una sorgente luminosa (laser) di opportuna lunghezza d'onda. L'immagine dell'intero oggetto viene ottenuta mediante scansione rapida e, ricostruita via *software*, da una successiva analisi del segnale fluorescente emergente dei singoli punti scansionati. Presso il CGS la microscopia confocale trova applicazioni in citologia, istologia, biologia cellulare fornendo informazioni morfologiche, morfometriche e spaziali, in fisiologia per lo studio sulla variazione della concentrazione di ioni cellulari (calcio, magnesio), per determinare le variazioni di pH cellulare nonché del potenziale delle varie membrane cellulari. La microscopia confocale ha inoltre indotto il perfezionamento e la messa a punto di tecniche come FRET (*Fluorescence Resonance Energy Transfer*), FRAP (*Fluorescence Recovery After Photobleaching*) e FLIP (*Fluorescence Loss in Photobleaching*) che forniscono informazioni sulla mobilità delle molecole all'interno delle cellule. L'elevata automatizzazione del sistema consente di acquisire immagini nel tempo (*Time lapse*) sia ad intervalli regolari che differenziati garantendo la possibilità di seguire *in vivo* fenomeni biologici.

Laboratorio di Cristallografia

Dott. Massimo Boiocchi (Tel. 0382.987531)

Il laboratorio è dotato di un diffrattometro a cristallo singolo a quattro cerchi ENRAF-NONIUS CAD4 con rilevatore puntuale e radiazione X incidente Mo-K α monocromatizzata con grafite. Il laboratorio ha un aggiornato software cristallografico e possiede una banca dati di circa 360.000 strutture. L'apparecchiatura consente di determinare la disposizione spaziale di atomi o ioni che costituiscono il campione, nello stato solido, di sostanze organiche, inorganiche, metallorganiche o di interesse biologico. La cristallografia può essere utile nell'analisi dei minerali (identificazione delle fasi mineralogiche e studi cristallografici, definizione delle relazioni fra struttura cristallina, ambiente di formazione, caratteristiche fisiche e potenzialità tecnologiche dei minerali delle rocce), nell'analisi delle molecole organiche, inorganiche o metallorganiche di sintesi (definizione della struttura della molecola ovvero della stereochimica e delle sue relazioni con il comportamento chimico delle sostanze, identificazione delle interazioni intramolecolari "chimica supramolecolare" e delle loro variazioni in funzione dell'ambiente chimico), nell'analisi dei farmaci (identificazione della struttura molecolare del principio attivo di un farmaco compresa la configurazione assoluta e della sua relazione con l'attività sui sistemi biologici), nell'analisi dei materiali (identificazione della relazione fra struttura alla scala atomica di un materiale e sue proprietà finali).

Laboratorio di Microscopia elettronica

Sig. Vittorio Necchi (Tel. 0382.987837)

Il laboratorio è dotato di un microscopio elettronico a trasmissione JEOL JEM-1200EXIII corredato di TEM CCD camera Mega View III e di strumentazione accessoria, come l'ultramicrotomo Reichert Ultracut S per il sezionamento dei campioni inclusi. Per l'utenza del Centro, la microscopia elettronica a trasmissione trova applicazione soprattutto in campo biologico, sia nell'ambito della ricerca che in quello diagnostico, e contribuisce in maniera importante alla conoscenza dei fenomeni fisiopatologici, oltre che alla diagnosi di alcune patologie; negli ultimi anni la Microscopia Elettronica a Trasmissione ha avuto impiego anche nella determinazione di dimensioni, morfologia, distribuzione dimensionale e aggregazione di nanoparticelle.

Laboratorio di Risonanze Magnetiche

Dott.ssa Laura Linati (Tel. 0382.98.7549)

Il Laboratorio è dotato di uno spettrometro a Risonanza Magnetica Nucleare (NMR) Bruker FT-NMR Avance400 basato su magnete superconduttore da 9,4 tesla, corrispondente ad una frequenza di eccitazione del nucleo ^1H di 400 MHz. Si tratta di una macchina multinucleare a trasformata di Fourier (FT-NMR) dedicata allo studio esclusivo dei liquidi in alta risoluzione. La strumentazione consente applicazioni della spettroscopia a risonanze magnetiche in campo chimico (caratterizzazione strutturale e dinamica di molecole), in campo ambientale (determinazione di inquinanti organici in varie matrici come acque, terreni, cibi), biomedico (dinamica e morfologia di tessuti, analisi di processi metabolici), farmaceutico (identificazione di impurità di sintesi e di degradazione nei farmaci, analisi dei metaboliti dei farmaci).

Laboratorio di Spettrometria di massa

Dott.ssa Barbara Mannucci (Tel. 0382.987532)

Il Laboratorio è dotato di due strumenti accoppiati a gascromatografi (GC/MS CombiPAL /Thermo Scientific DSQ II Single Quadrupole e GC/MS Thermo Scientific DSQ II Single Quadrupole) e di due spettrometri di massa accoppiati a cromatografi liquidi (LC/MS Thermo Scientific LCQ Fleet Ion Trap e LC/MS Thermo Scientific LTQ Linear Ion Trap). La strumentazione è utilizzata per la determinazione di strutture chimiche e per la rivelazione qualitativa e quantitativa di sostanze organiche in miscele complesse. La disponibilità di strumentazione con caratteristiche differenti rende il laboratorio attivo in molti campi tra cui i settori dell'analisi ambientale (determinazione di inquinanti organici in varie matrici come acque, terreni, cibi), dell'analisi dei farmaci (identificazione e determinazione quantitativa di impurezze di sintesi e di degradazione nei farmaci, ed analisi dei loro metaboliti), dell'analisi dei cosmetici (determinazione di sostanze allergeniche nei cosmetici e negli estratti naturali), dell'analisi di biomolecole (identificazione e caratterizzazione strutturale di peptidi e proteine), della medicina legale (determinazione di farmaci/droghe, anche in fluidi e tessuti biologici).

Laboratorio di Spettroscopie

Dott. Federica Corana (Tel. 0382.987529)

Attualmente il laboratorio serve di supporto per problematiche strutturali e conformazionali di molecole chirali, inclusi i composti metallo-organici e le proteine. Il laboratorio è dotato di diversi strumenti: uno spettropolarimetro Jasco J-710 per la determinazione del dicroismo circolare di molecole semplici e di macromolecole, dotato di accessorio per misure cinetiche veloci, uno spettrofluorimetro Perkin elmer LS50 B per la misura degli spettri di emissione di sostanze fluorescenti in soluzione e in matrice, ed uno spettrofluorimetro a singolo conteggio di fotone (Edinburgh Analytical Instruments) ad alta sensibilità, per la determinazione di tempi di vita degli stati eccitati con possibilità di condurre misure a temperatura variabile.

Laboratorio per la Struttura primaria delle proteine

Dott.ssa Patrizia Arcidiaco (Tel. 0382.987260)

Il sequenziatore automatico Hewlett Packard modello Protein Sequencer G1000A collegato ad un elaboratore dati HP Kayak P III 500 MHz consente di eseguire l'analisi della sequenza N-terminale di proteine e peptidi, operando su piccole quantità di campione (100-150 pmoli). Il sistema di analisi si basa sulla reazione di Edman. L'identificazione del derivato di ogni singolo amminoacido (PTH) viene eseguita mediante cromatografia liquida in fase inversa (RP-HPLC) per confronto del suo tempo di ritenzione con quelli di una miscela standard. Inoltre il laboratorio è dotato di due analizzatori di amminoacidi (HP Amino Quant serie II 1090L collegato ad un Pentium III e Jasco X-LC con rilevatore a fluorescenza collegato a Pentium IV) che permettono la determinazione qualitativa e quantitativa degli amminoacidi liberi di idrolizzati proteici e di campioni biologici. L'attività del laboratorio riguarda principalmente la verifica della sequenza di proteine purificate da fonti naturali o prodotte in forma ricombinante, l'identificazione di proteine incognite, la determinazione della composizione amminoacidica di peptidi o proteine, l'analisi di farmaci di tipo peptidico, l'analisi di integratori alimentari a base amminoacidica-proteica-peptidica.

Observation and analysis of flat-lying molecular layer via scanning probe microscopy

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Summary

The analysis of the formation of ultra-thin organic films is a very important issue. In fact, it is known that the properties of organic field effect transistors are strongly affected by the early growth stages. For instance, in the case of sexithiophene deposited on thermal SiO_x, the presence of domains made of molecules with the backbone parallel to the substrate surface has been indirectly evidenced by photoluminescence spectroscopy and directly imaged by Ultrasonic Force Microscopy. On the contrary, photoluminescence spectroscopy have failed to observe sexithiophene deposited on native SiO_x. In this paper, we show how Scanning Probe Microscopy techniques combined with post-annealing process are able to identify a flat-lying molecular layer on the native SiO_x. By using Lateral Force Microscopy and Atomic Force Microscopy in Intermittent Contact we investigate its mechanical properties and, by using Ultrasonic Force Microscopy, we directly identify the structure of flat-lying molecular layer. Finally, we show how the post annealing process can address the growth of organic materials towards a "driven layer-by-layer growth" to both improve the surface coverage and reduce the domain boundaries effects in organic field effect transistor.

Key words: scanning probe microscopy, organic growth, α -Sexithiophene, flat-Lying molecules, annealing.

Introduction

In organic electronics, conjugated oligomers represent an important class of molecules besides conjugated polymers (Malliaras, 2005). While polymers are generally more amenable to processing, the oligomers, having lower molecular weight, can be deposited in a much more controlled fashion down to the molecular scale. As a consequence, the oligomer film morphology is easier to characterize and to relate with physical properties such as molecular conformation and organization, crystallinity, grain size and orientation (Pope, 1998). This provides an important path to identify and explore key issues necessary for improving organic electronic properties such as charge conductivity and recombination (Fraboni, 2009). In particular, the interface between the organic film and the dielectric substrate has turned out to be vital for the performances of

organic transistors and the morphological properties of the first Monolayer (ML)* have been correlated to the conduction properties of these devices (Dinelli, 2004 a, b). This is particularly important for the fabrication of electroluminescent devices where it is necessary to combine two organic films providing, respectively, paths for electron and hole conduction (Hepp, 2003).

Therefore, in the past few years, the initial growth stages (i.e. at a sub-monolayer coverage) have been intensively studied by both X-ray Diffraction (XRD) and Atomic Force Microscopy (AFM). Whereas it has provided accurate details on the crystallinity (i.e. unit cell size and molecular orientation) and grain size distribution, XRD is applicable to relatively thick samples when at least the first ML is complete (Moulin, 2006).

*A coverage of one Monolayer (1 ML) is defined as the amount of material needed to cover the substrate with one layer of T6 molecules having the backbone perpendicular to the surface.

On the other hand, AFM represents the key approach linking XRD data and film morphology in the real space, as well as providing access to local information with nanometer scale spatial resolution rather than averaged over a large sample area (Annibale, 2007).

In particular, when conjugated oligomers are deposited on chemically inert and flat SiO_x surfaces, firstly form stable nucleus of few molecules. Subsequently, nucleus evolve as islands during the deposition and coalesce one each other to form a continuous organic layers (Wu, 2007). The film growth is characterized by two contemporaneous molecular configurations: the upright configuration of the islands and the flat-lying configuration in between of them. During the film's growth, these flat-lying molecules can affect islands coalescence in two ways: if they remain flat, an empty space in between islands is created; otherwise, if they are collected from islands, the interfacial disorder between islands is increased (domain boundaries).

These flat-lying molecules are observed in the case of sexithiophene (T6) sub-monolayer films deposited on thermal silicon dioxide (SiO₂) surfaces. Photoluminescence spectroscopy (PS) has revealed crucial information on the molecular organization allowing to identify a significant population of domains made of T6 molecules lying flat in direct contact with the substrate (Loi, 2005). PS has allowed for the first time to provide insight into the initial growth stages of organic films in reason of the T6 J-aggregates formed on thermal SiO₂ (Da Como, 2006). Authors observed that besides the upright configuration with molecular axis normal to the sample surface, T6 molecules can also arrange themselves in a flat-lying configuration. Recently, these T6 flat-lying aggregates on thermal SiO₂ are directly imaged by Ultrasonic Force Microscopy (UFM) (Dinelli, 2011).

To extend the T6 J-aggregates investigations, PS measurements have been repeated on chemically treated SiO_x to correlating it with the surface physical/chemical properties (Dinelli, 2006). PS had failed to image flat-lying molecules on native SiO_x. This experimental result allows to infer that such T6 aggregates are formed in reason of the hydrophobic nature of the thermal SiO₂. In reason of the result, further investigation are necessary to investigate how T6 molecules are organized on the native SiO_x.

In this paper, we present an investigation carried out by means Scanning Probe Microscopy (SPM) techniques to identify and analyse the T6 flat-lying aggregates when T6 molecules are deposited on native SiO_x. In particular, T6 films in sub-monolayer regime are investigated by using AFM operating in Contact Mode (CM), Intermittent Contact Mode (ICM) and in the Ultrasonic Force Microscopy (UFM) configuration (Kolosov, 1993). By controlling carefully the experimental conditions of the growth, we have indirectly observed the physical properties of an homogenous layer of flat-lying molecules which covers the native SiO_x surface in between T6 islands. In addition, this homogenous layer can be re-organized through a short post-annealing process (not exceeding 1 hour). When a sub-monolayer film is deposited on a heated substrate, this process supplies exceeding energy able to move flat-lying molecules. In reason of their weak bond with the substrate they can move towards islands (which are energetically favoured) increasing the island's size. In such way we have realized a *quasi*-complete monolayer film.

Materials and Methods

T6 molecules, purchased in powder from Sigma Aldrich[®], were vacuum sublimated on Si substrates (*p*-type, $\rho=2 - 10\Omega\text{cm}$) with native SiO_x. The amount of molecules deposited on the SiO_x substrate and the deposition rate *r* were kept constant to 50 ng and 5 ng/min, respectively. They are measured through a Quartz Micro-Balance (QMB) placed near to the substrate to monitor the amount of deposited molecules with 1ng of sensitivity. To avoid thermal drift effects in the QMB measurements, it is cooled by a water cooler to keep its temperature constant (20°C). This sensitivity allow us to estimate the surface coverage with an error lower than the 1%. T6 molecules deposited on SiO_x substrates form rounded islands covering approximately the 30% of the SiO_x surface (sub-monolayer regime).

To implement the post-annealing process, films are grown with the substrate kept to 80°C and 100°C. These temperature are chosen in reason of the negligible amount of molecules re-evaporated from the heated substrate ("complete condensation" regime) (Venables, 1984). Conversely, for

120°C the 90% of the total deposited molecules are re-evaporated, so this temperature is useless for our research aims. After the molecules deposition, the substrate is kept for 1 hour at the same temperature (post-annealing process – 80°C or 100°C), while the sample and the Knudsen cell shutters are closed. In this period, the temperature of the T6 powder in the Knudsen cell is decreased from 230°C to 180°C, to avoid the sublimation of molecules. After the post-annealing process, the substrate has been cooled to RT with the same temperature decay time (approx. 10 minutes) and removed from vacuum through a Fast Entry Lock (FEL).

The morphologies of sub-monolayer T6 films and the mechanical properties of the flat-lying molecular layer have been observed by using several SPM techniques: 1) ICM used in attractive and repulsive regimes (Garcia, 2002); 2) CM measuring the later deflection of the cantilever, namely Lateral Force Microscopy (LFM) (Sundararajan, 2000); 3) UFM which is a technique based on a standard AFM operating in CM with the additional application of an ultrasonic vibration to the substrate, well above the AFM cantilever resonance. In this way, the friction force can be eliminated because the tip-sample contact is broken several times while the tip is laterally moved during the imaging process (Dinelli, 1997).

Results and Discussions

In order to investigate the flat-lying molecular layer, firstly we study the effect of the post-annealing process. The averaged surface coverages $\langle\Theta\rangle$ has been calculated for each temperature (80°C and 100°C) and without (or with) the post-annealing process. We define $\langle\Theta\rangle$ as the mathematical average of three coverages measured from three AFM topographic images (20 μm x 20 μm) of three different surface regions. Figure 1 shows the $\langle\Theta\rangle$ results.

The statistical analysis of $\langle\Theta\rangle$ shows clearly that the post-annealing process increase the islands size. For 80°C, $\langle\Theta\rangle$ (expressed in percentage of the surface covered by T6 islands) runs from 33% to 36% (+3%), while for 100°C it increases from 28% to 34% (+6%). $\langle\Theta\rangle$ values for samples grown at 80°C and 100°C (without post-annealing) suggest a slightly re-evaporation of T6 molecules during the deposition (-6%) but can be considered

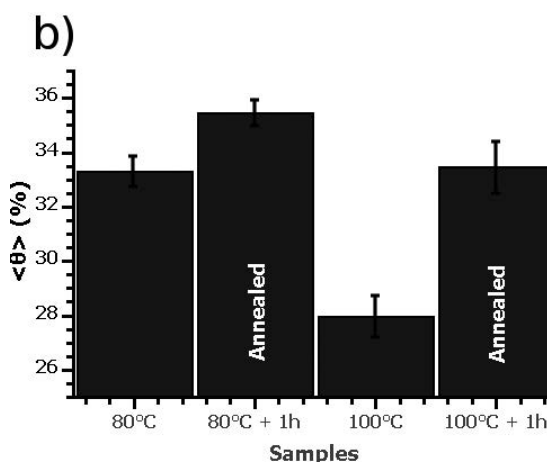
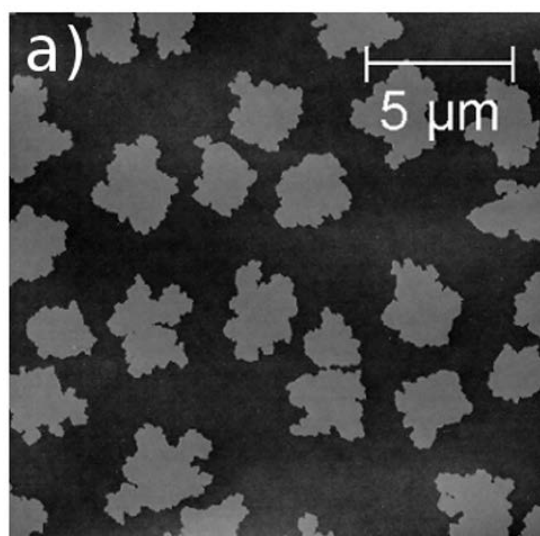


Figure 1. a) AFM topographic image of T6 islands grown on native SiO_x . Islands are red labelled through the threshold process by means of islands are selected through their height (upright molecules are 24 Å long) and $\langle\Theta\rangle$ is calculated. b) Averaged surface coverage $\langle\Theta\rangle$ (in percentage) vs. the sample type. A coverage of 100% corresponds to a surface completely covered by upright T6 molecules.

negligible respect to the 90% observed for 120°C.

These results indicate that some molecules (not visible in AFM topographic image) are present in between islands and they are re-organized through the post-annealing process. In particular, the heat (energy) provided 1 hour after the deposition gives energy and time to move the not-organized molecules towards islands thus increasing their size. This is particularly evident in the 100°C case, where the size is increased up to 6% while, in the 80°C case, molecules have not enough energy to be collected by islands (just 3% more).

The surface coverage $\langle\Theta\rangle$ is an indirect meas-

urement of the re-organization effect induced by the post-annealing process. Now, it is important to turn out these experimental observation by using SPM techniques which give high spatial resolution and local physical information. Assuming a flat-lying molecular layer in between islands (even after the post-annealing process), it is clear that a molecular layer on bare SiO_x change its friction property.

To investigate friction we used LFM technique that is extensively used to study friction of various samples with ultralow loads (few nN) and scan range from tens of micrometer down to the atomic scales. In general, the LFM technique is a contact technique, i.e. the tip is permanently in contact with the surface. During the surface scan, the microscope feedback system use the vertical movements induced by the cantilever deflection (laser spot is moved up and down on the photodiode – Normal forces F_n) as signal for measuring the surface topography. At the same time, due to the friction forces F_f between the tip and the surface, the cantilever is slightly twisted (friction signal). The cantilever torsion is measured by the lateral movements (left and right) of the spot on the photodiode. During friction measurements, the friction signal from both forward and backward scans are needed in order to understand the origins of the observed friction forces. It is well known that when an AFM tip is scanned across a sample surface, the measured friction forces (or lateral forces) are generated by both material effects as well as topography-induced effects (Sundararajan, 2000). Those effects are independent to the scan direction, hence they are eliminated subtracting the friction data of the backward scan from that of the forward scan, leaving only material-induced effects (friction forces) (Muller, 1997).

The surface friction is well described by the friction coefficient μ , which is a dimensionless scalar value that describes the ratio of the force of friction between two bodies (F_f) and the force pressing them together (F_n). In general, $\mu = \mu_s + \mu_k$ but the static friction is usually larger than its kinetic counterpart, so $\mu \approx \mu_s$. In first approximation, F_f and F_n are proportional to the torsion and the deflection differences (as measured from the photodiode - nA). The deflection difference (ΔD) is measured as the difference between the cantilever deflection when the tip doesn't interact with the surface and the set-point deflection. On the other hand, the torsion difference (ΔT) is measured as

the difference between the lateral position of the laser spot when the tip doesn't interact with the surface and the statistical torsion values (Gaussian distribution) measured from the lateral force images. For our measurements we used soft cantilever (NT-MDT CSG10, $k = 0.11\text{N/m}$) and we apply low load (4 nN at most). In such conditions, we can evaluate the friction coefficient as $\mu^* = F_f$

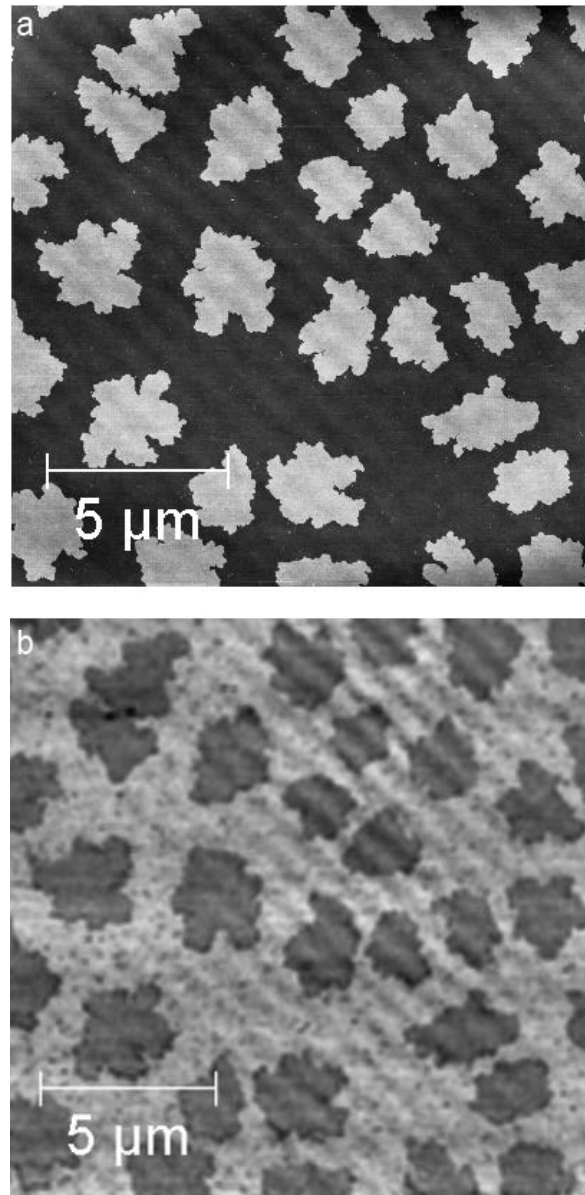


Figure 2. a) AFM topographic image of T6 islands grown on native SiO_x . b) Friction image obtained by subtracting the friction data of the backward scan from that of the forward scan.

$/F_n \propto \Delta T/\Delta D$, where μ^* indicates the friction coefficient when the cantilevers are not calibrated. It is useful to study the trends the surface's friction vs. substrate temperature and post-annealing process. Figure 2 shows the friction image subtracting friction data in backward and forward scans. Topographical artefacts were eliminated and two regions are clearly visible: bright regions (substrate) and dark regions (T6 islands). Afterward, μ^* has been evaluated from the statistical analysis of friction images of T6 samples compared to the friction image of bare SiO_x (Figure 3).

The evolution of friction coefficient confirms the presence of a flat-lying molecular layer which increase the friction force between tip and sample. As expected, the sample grown at 80°C shows higher friction force (lower thermal energy, higher amount of molecules in between islands), that decreases with the increases of the substrate temperature and the post-annealing process. This trend sustains two hypothesis: a) an homogenous flat-lying molecular layer exists in between islands; b) the post-annealing process promote the re-organization of these molecules. Notably, the sample grown at 100°C , and annealed for 1 hour, shows the same friction coefficient of the bare SiO_x suggesting a complete re-organization of the molecules.

In order to both confirm the results obtained up to now and reach high spatial resolution images, we performed AFM measurements in ICM. It is a less invasive technique respect to the CM technique so, in principle, a proper setting of the work-

ing conditions for phase contrast in ICM should allow one to identify the flat-lying molecular layer with high spatial resolution. When ICM works with a high amplitude set-point (i.e. small reduction of the free cantilever oscillation amplitude, namely attractive regime (Garcia, 2002)), the averaged tip-sample force $\langle F_{ts} \rangle$ is about a few hundreds of pN (Holsher, 2006). On the contrary, when the amplitude set-point is decreased, $\langle F_{ts} \rangle$ is increased up to 20 nN (namely repulsive regime). Figure 4 shows a topographic and phase images in attractive (A) and repulsive (R) regimes for the 80°C sample (the sample with the higher amount of molecules in between islands).

By looking the phase images in attractive regime, the substrate shows a modulation of the contrast as if it be composed by mounds. Conversely, in repulsive regime the contrast is flattened and mounds are replaced by dark regions rounded by brighter edges. This qualitative observation can be investigated by comparing the average roughness R_q of the interstitial regions surrounding the T6 islands measured in both regimes. In attractive regime, $\langle F_{ts} \rangle$ is about 400 pN and R_q is (2.7 ± 0.5) Å while in repulsive regime, where $\langle F_{ts} \rangle$ is about 16 nN, R_q become (1.9 ± 0.3) Å. This R_q variation indicates the presence of a compliant molecular layer on the SiO_x substrate, which can be compressed when $\langle F_{ts} \rangle$ increases. The presence of this compressible layer is also confirmed by the height distribution measured on the substrate in both regimes (Figure 5a – up). The height peak shift of 2.5 Å passing from attractive to repulsive regime. In repulsive regime the number of counts for lower height is notably increased to represent a surface flattening when $\langle F_{ts} \rangle$ increases. As comparison we have measured the height distribution in both regime for bare SiO_x (Figure 5a – down). Here, the height peak shifts less (1 Å) therefore a variation of 2.5 Å is understandable only if we consider the surface coated by a soft and compressible layer.

As shown in Figure 4, for ICM we have also measured the shift of the cantilever oscillation. Its absolute value shows the operating regimes: Attractive (phase value $>90^\circ$) or Repulsive (phase value $<90^\circ$) (Garcia 2002). However, even in the phase contrast no clear structure appears in the images, suggesting that the flat-lying molecular layer is homogeneously distributed. As described by Garcia (Garcia 2002), the phase is correlated to the energy provided by the tip and dissipated into

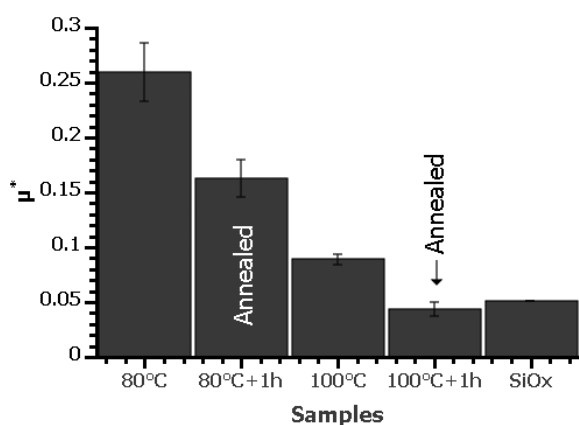


Figure 3. Evolution of the friction coefficient μ^* vs. substrate temperature and post-annealing process, compared to the bare SiO_x .

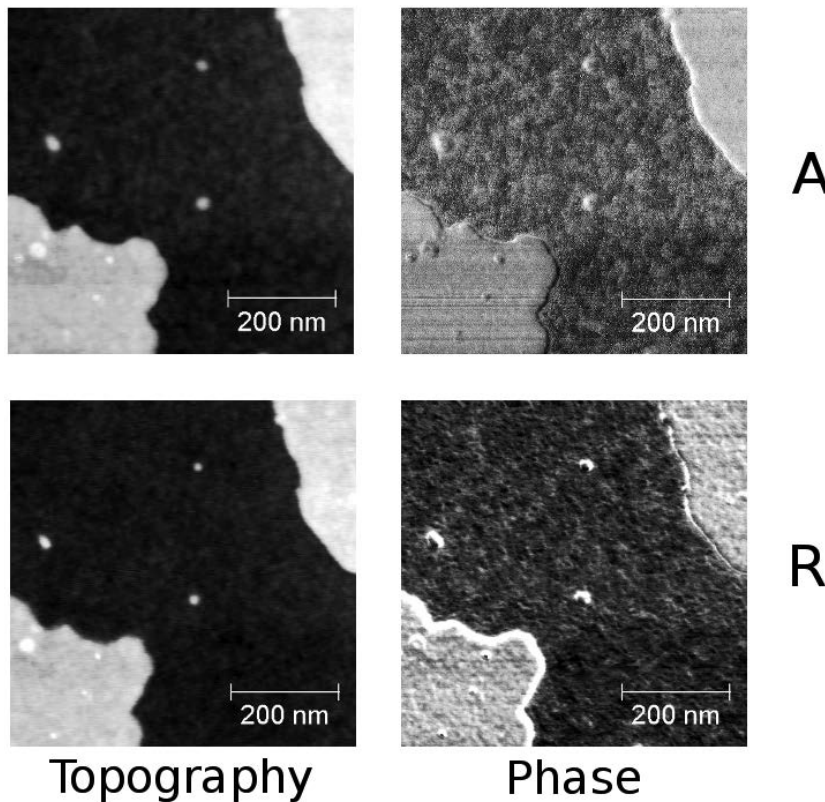


Figure 4. AFM topographic (left) and phase (right) images in attractive (A-up) and repulsive (R-down) regimes. In the attractive regime, the tip interacts with the surface through the van der Waals forces. On the contrary, in repulsive regimes it interacts through the Hertz repulsion forces.

the contact region of the surface, through the following relationship:

$$E_{diss} \approx \frac{\pi \cdot k \cdot A_{SP}}{Q} \cdot (A_0 \sin \phi - A_{SP})$$

where k and Q are the elastic constant and the quality factor of the cantilever, respectively, A_0 is the free amplitude oscillation, A_{SP} is the set-point amplitude oscillation and ϕ is the phase value.

As shown in Figure 5b, the energy dissipated into the substrate increases with the increasing of the substrate temperature and the post-annealing process. This trend is in agreement with the presence of a soft layer over the SiO_x , i.e. less damping of the cantilever oscillation (less energy dissipated) is expected for softer samples (with Young's modulus $E \leq 10 \text{ GPa}$) (Tamayo, 1996). The high dissipated energy onto the sample grown at 100°C of substrate's temperature and post-annealed for 1 hour, validates this observation because the AFM tip interacts with the bare silicon oxide ($E \approx$

130 GPa). The same energy trend is observed for attractive and repulsive regimes where the tip interacts with the sample through the van der Waals force and the Hertz repulsive force, respectively (Tamayo, 1996). In the repulsive regime, cantilever's damping is due to the viscoelastic properties of the material so, with the re-organization of the molecular layer, the energy dissipated (damping) is increased. The same behaviour is observed in attractive regime where the long-range range adhesion hysteresis is responsible to the high dissipated energy (Gomez, 2010).

CM and ICM have shown indirectly the presence of a flat-lying molecular layer and its mechanical properties, but they are too much invasive to image it with high spatial resolution. For such reason we used Ultrasonic Force Microscopy (UFM) (Kolosov, 1993) that provides both the nanometer scale spatial resolution and the material sensitivity necessary to identify both upright and flat-lying molecular layer in the initial stages of T6 film growth. It is based on a standard AFM operating in

CM with the additional application of an ultrasonic vibration to the substrate by means a modified sample holder. It is composed by a piezoelectric disk with resonant frequency of 2MHz, glued to a standard metallic sample holder. The samples under investigations are then directly attached to the top of the piezoelectric disc via a thin layer of ultrasonically transparent and replaceable binder,

namely phenylsalicilate. Ultrasonic vibration (2MHz) is well above the AFM cantilever resonance. In this way, the friction force can be eliminated because the tip-sample contact is broken several times while the tip is laterally moved during the imaging process. Any possible damage to the sample or the tip is thus minimized. Additionally, UFM is material sensitive (Briggs, 2010), so we are able to differentiate where the flat-lying molecular layer isn't homogenous, imaging it with unprecedented spatial resolution.

Figure 6 shows topographic (a-left) and ultrasonic (a-right) images of the sample grown with 80°C of substrate temperature. The ultrasonic contrast on the substrate is homogeneous (most of the surface is bright) besides some dark spots uniformly distributed. By zooming on a SiO_x surface region where no topographical features are present (Figure 6b-left), it is clear that the surface is covered by an homogenous layer (gray coloured) with black spots (two of them are indicated by white arrows). This observation is confirmed by the statistical measurements of the contrast on that region (Figure 6b-right); it is a convolution of two Gaussian distributions, one centred on (5.14±0.12)V (lower statistical weight) and the other one centred on (5.26±0.12)V (higher statistical weight). In reason of the high material sensitivity of the UFM technique, that contrast can be associated to the SiO_x substrate (lower value) and to the flat-lying molecular layer (higher value).

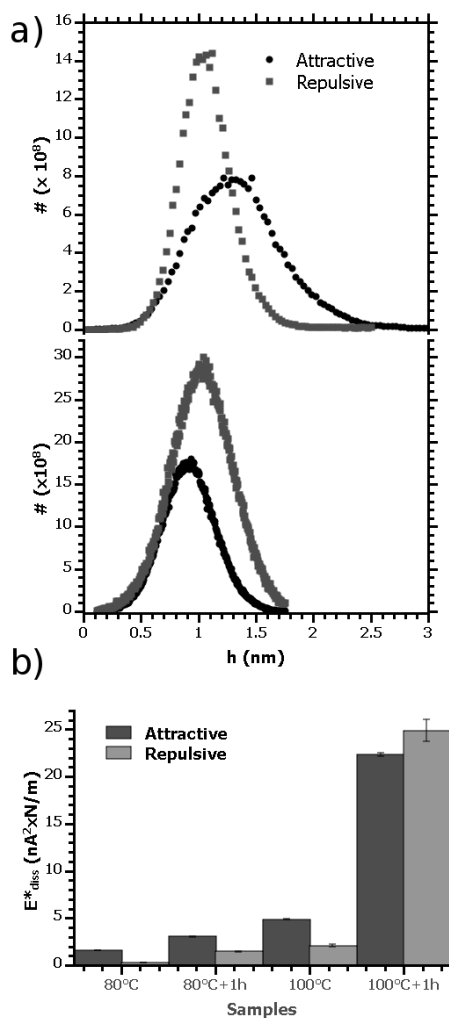


Figure 5. a) Cumulative height plots for the sample grown at 80°C (up) and the bare SiO_x (down). The height peak correspondent to the substrate is shifted when the tip-sample interaction pass from the attractive (black spots) to the repulsive (grey spots) regime. b) Energy dissipated vs. substrate temperature and post-annealing process where E^*_{diss} indicates the energy dissipation for not-calibrated cantilevers, as described for the friction coefficient.

Table 1. Ultrasonic Deflection ud (in V) measured on both the substrate and the T6 islands, and their difference. An increase of the ud difference value corresponds to a more stiff substrate. In fact, the calculated difference (last column) shows that the post-annealing process free the substrate from the flat-lying molecular layer increasing the ud difference. All measurements were made with cantilevers with spring constant of 0.9 N/m, with the exception of the measurement signed by * which is done with a cantilever stiffer (3N/m). To be compare with the other measured, the measured ud value should be multiply roughly for the spring constants ratio, i.e. 3/0.9.

Samples	Substrate (V)	6T islands (V)	Difference (V)
80°C → RT	5.4748	4.1666	1.3082
80°C → 1h → RT	3.0214	2.5415	1.4397*
100°C → RT	1.1389	0.7326	0.4063
100°C → 1h → RT	1.5230	0.9622	0.5608

Besides the sample grown at 80°C, all the other samples are investigated by UFM. Table 1 summarizes the obtained results which confirm what observed by using CM and ICM: a flat-lying molecular layer in the interstitial regions between T6 islands exist and it is re-organized by means the post-annealing process.

Conclusions

T6 films with sub-monolayer coverage are grown with different substrate temperature (80°C and 100°C) and constant amount of deposited molecules. Afterwards, they are submitted (or not submitted) to a post-annealing process.

Samples are investigated by using Scanning Probe Microscopy techniques. They have shown that a flat-lying molecular layer exists on the SiO_x surface and it can be re-organized by means the post annealing process. It shows an homogenous distribution of flat-lying molecules compared to the mound distributions observed on thermal SiO_x. This suggest that the PS contrast elsewhere observed is due the hydrophobic nature of the thermal SiO_x. In addition, the sample grown at 100°C

and post-annealed for 1 h have shown a complete molecular re-organization as proved by the decreasing of the friction force (LFM), the increasing of the dissipated energy (ICM) and the material contrast observed by ultrasonic contrast.

In perspective, this method can address the growth of organic materials towards a “driven layer-by-layer growth” which will be able to both improve the surface coverage and reduce the domain boundaries effects (in reason of the improved molecular organization) which are detrimental for the electrical performances of the organic devices.

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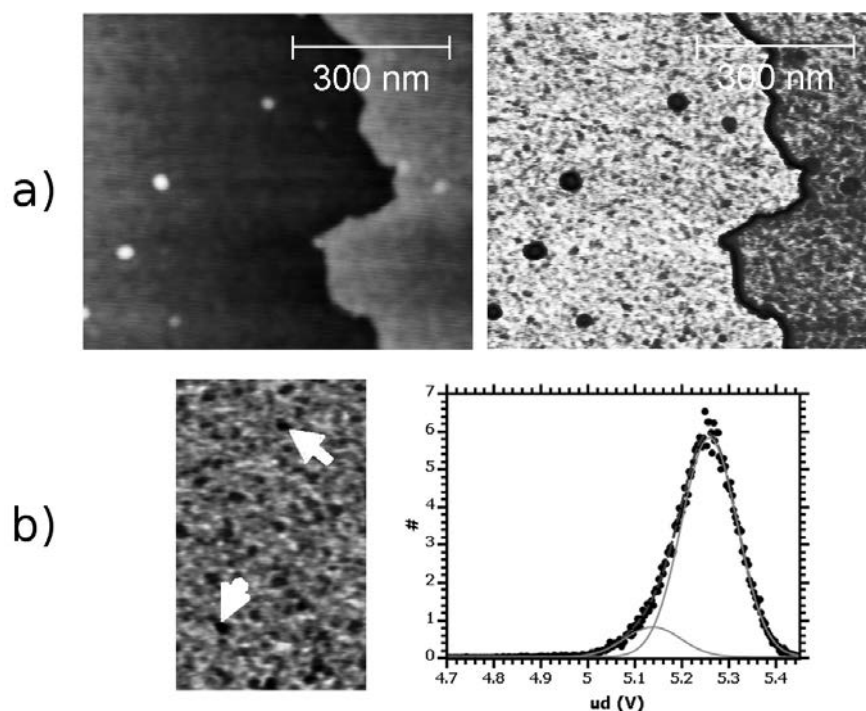


Figure 6. a) Topographic (left) and ultrasonic (right) images of the 80°C sample; b) Ultrasonic image of a substrate region free of topographic features (left) and correspondent statistical distribution of the ultrasonic deflection ud (expressed in volts) that is induced on the cantilever by the piezoelectric plate (right). White arrows show two dark spots correspondent to the SiO_x substrate.

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Cell death induced by physical agents: morphological features

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Summary

The aim of this work is to present and discuss in vitro cell death appearing after exposure to physical conditions such as UVB radiation, static magnetic fields, hyperthermia and hypothermia. UVB radiation induces oxidative stress, leading, in most experimental models, to apoptotic death. Generally death occurs through the intrinsic apoptotic pathway, even if the extrinsic one cannot be excluded. UVB radiation also appears effective on cell systems which are normally apoptosis-resistant, such as muscle cells. Static magnetic fields mostly induce plasma membrane and microvilli alterations; occasionally apoptotic cell death appears. Hyperthermic conditions applied were mild, i.e. variable exposures to 43°C, as well as hypothermia, consisting of variable exposures to 0-6°C. Both treatments were followed by incubation at physiological conditions. Heat exposure is a powerful apoptotic inducer in a variety of cells, where it induces classical apoptotic changes and the well known biochemical pathways. The effect of hyperthermia has been described in adherent human tumor cells, which undergo cell rounding and progressively detach from the substrate, in close correlation with the down-regulation of adhesion molecules. Hypothermia, only occasionally triggers apoptosis, more frequently inducing cell necrosis. Therefore, cell death can be induced by physical agents dependently on the treatment and cell model. In particular, UVB and hyperthermia can be considered reliable and reproducible apoptotic triggers.

Key words: apoptosis, UV-B irradiations, magnetic fields, hyperthermia, hypothermia.

Introduction

Apoptosis plays a pivotal role in the deletion of unwanted, damaged, or infected cells in multicellular organisms, as well as in development and tissue homeostasis, cell differentiation, and proliferation (Ola *et al.*, 2011). It is a highly regulated form of cell death, the dysregulation of which results in pathological conditions including cancer, autoimmune disorders and neurodegenerative diseases. It is known in literature that various physical agents are possible apoptotic inducers, but, in some cases, their mechanism of action is only partially known. Some of them, however, are frequently used in clinical trials and represent a potential

apoptogenic tool, which requires a major consideration both in terms of basic and applied biomedical research. Ultraviolet (UV radiations) in the middle-wavelength range, between 290 and 320 nm (UV-B), represent a relevant environmental danger because of their role in skin aging and cancer (Lei *et al.*, 2010), as well as in the exacerbation of infections (Srivastava *et al.*, 2008). UV-B radiation is a potent apoptosis inducer in many cell types (Luchetti *et al.*, 2006; Liu *et al.*, 2007; Pozzi *et al.*, 2007; Paz *et al.*, 2008) and also in muscle cells (Salucci *et al.*, 2010). It can induce both extrinsic and intrinsic apoptotic pathways, but it is still unclear how these pathways are interrelated (Sandri *et al.*, 2001). However, DNA damage is a

crucial event in UV-B-mediated apoptosis (Burattini *et al.*, 2009). Morphological observations showed that low doses of UV induced apoptosis (Caricchio *et al.*, 2003; D'Emilio *et al.*, 2010), whereas higher doses triggered both apoptosis and necrosis (Abu-Yousif *et al.*, 2008). Static magnetic fields (SMF) might cause cancer or many other human health problems, but there is very little laboratory or epidemiological evidence that connects SMF exposure and human health hazards. There have been few studies on the effects of static magnetic fields at the cellular level, compared to those of extremely low frequency magnetic fields. Past literature showed that a static magnetic field alone has no lethal effect on cell growth or survival under normal culture conditions. It has also been reported that cell cycle is not influenced by extremely strong static magnetic fields (up to a maximum of 10 T). A further area of interest is whether static magnetic fields cause DNA damage, and/or micronuclei formation (Miyakoshi *et al.*, 2005). With the advent and vast use of diagnostic instrumentations such as NMR and the generation of extremely low frequency electromagnetic fields from common electrical devices, many sources of SMF have been introduced into our living environment (Franco *et al.*, 2008).

An increasing amount of evidences indicates that SMF can induce apoptotic changes, mainly through a modulation of Ca^{2+} influx (Teodori *et al.*, 2006). Exposure of cells to hyperthermia is known to induce apoptosis, although the underlying mechanisms are only partially understood. It has become a useful strategy, as well as surgery, chemotherapy, radiation and biological therapy in the fight against cancer. It plays an important role in multidisciplinary treatments (Harima *et al.*, 2001; De Hass-Kock *et al.*, 2009), representing a very promising method that will be seriously considered in the near future (Liang *et al.*, 2007). Hyperthermia-induced apoptosis is characterized both by its distinctive ultrastructural features and by the occurrence of internucleosomal DNA cleavage (Burattini *et al.*, 2009). The scientific basis of hyperthermia treatment is relatively known and its mechanisms of action seem to be multifactorial (Aravindan *et al.*, 2009). In the treatment of malignancies, hyperthermia was shown to induce in vitro both necrosis and apoptosis in a temperature-dependent manner (Sakaguchi *et al.*, 1995). Different experimental studies have demonstrated that it is an effective apoptotic trigger, favouring,

through integrin modifications, confirmed by flow cytometry adherent human cancer cell rounding and detachment from the substrate. Therefore, changes in cell adhesion properties, together with apoptosis induction were discussed and a potential role of hyperthermia in the control of metastatic diffusion was hypothesized (Luchetti *et al.*, 2004). Mild hypothermia protects cardiomyocytes undergoing oxidative stress by preventing apoptosis via inhibiting mitochondrial dysfunction and DNA damage (Lin *et al.*, 2010; Diestel *et al.*, 2010). It reduces myocardial damage and dysfunction after cardiopulmonary recovery correlated to a reduced rate of apoptosis and pro-inflammatory cytokine expression (Meybohm *et al.*, 2009). It protects the functions of brain cells from ischemic impairment by attenuating apoptotic death (Saito *et al.*, 2010; Li and Wang, 2011). Cells treated for 0-90 min at 0°C exhibited an exponential survival curve: thus, even short exposures to the cold (e.g. 5 min) produced measurable cell killing. Apoptosis represents a new manifestation of cell injury after brief exposure to 0-6 °C hypothermia in different cell lines (Columbaro *et al.*, 1995). The purpose of this work is to examine and discuss the morphological apoptotic effects of UV-B radiations, static magnetic fields, hyperthermia, hypothermia.

Materials and Methods

Cell Culture

Mouse C2C12 myoblasts were grown in flasks or on coverslips (in dishes), in the presence of DMEM supplemented with 10% heat-inactivated Fetal Bovine Serum, 2 mM glutamine at 37° C and 5% CO₂. To induce myogenic differentiation, when 80-100% cell confluence was obtained, previous medium was changed with DMEM supplemented with 1% FBS (D'Emilio *et al.*, 2010; Salucci *et al.*, 2010). Promyelocytic leukemia HL60 cells, myelomonocytic leukemia U937 cells, T-lymphoblastoid Molt-4, erythromyeloblastoid leukemia K562 cells, mastocytoma P815 cells and neuroblastoma SK-N-MC cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 25 mM HEPES pH 7.5, and 1% antibiotics. They were maintained at 37°C in humidified air with 5% CO₂ and cell viability was assessed by the trypan blue exclusion test

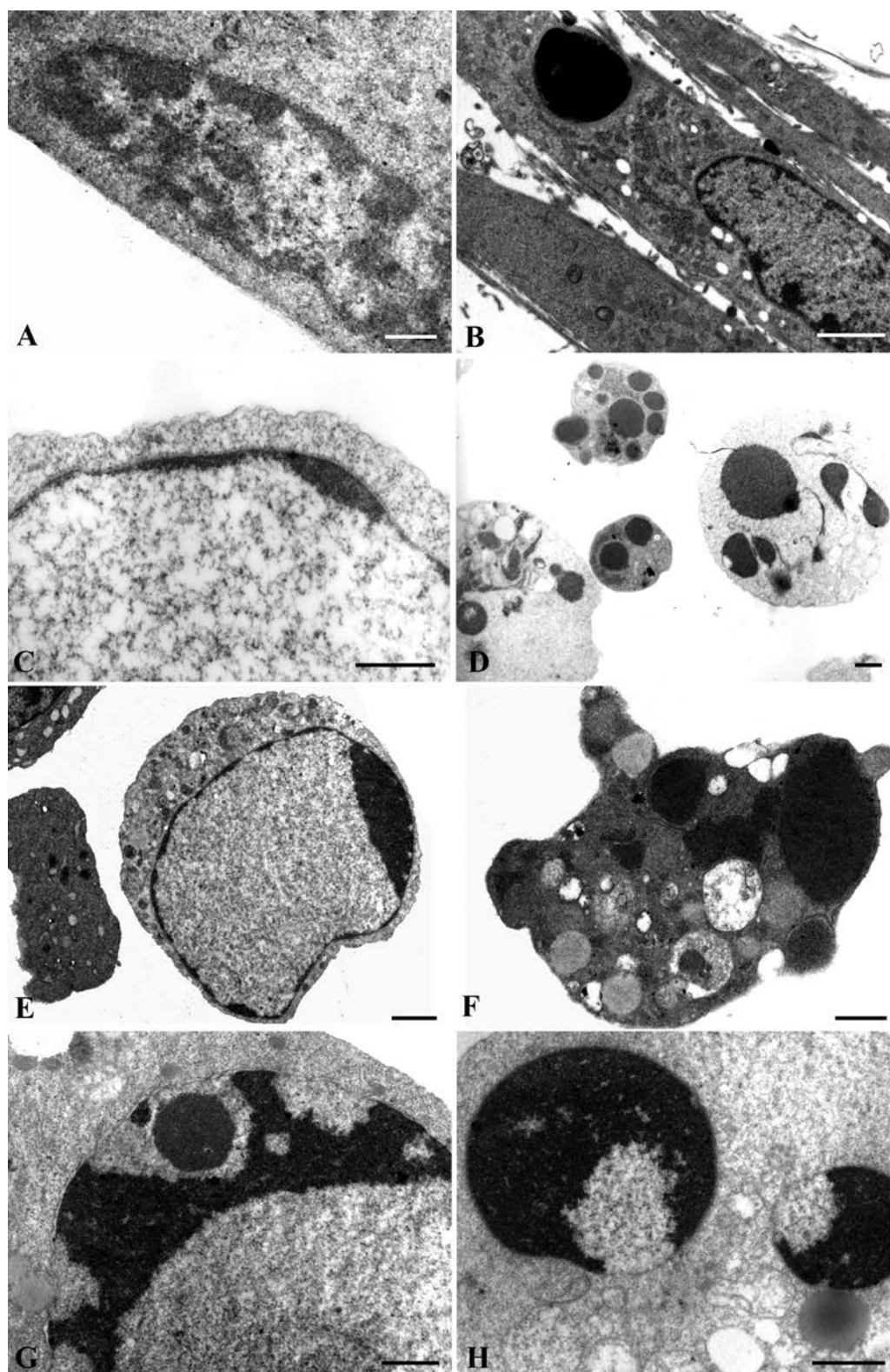


Figure 1. TEM of UVB-treated C2C12 (A, B), HL-60 (C, D), U937 (E, F) and MOLT-4 cells (G, H). All show chromatin margination (A, C, E, G), followed by nuclear splitting and micronuclei appearance (B, D, F, H). A, Bar = 1 μ m; B, Bar = 10 μ m; C, D, E, F, G, Bar = 0.5 μ m.

(Mariani *et al.*, Luchetti *et al.*, 2002; Sestili *et al.*, 2006). Glioblastoma cells were grown in a RPMI medium supplemented with 10% FCS. For the experimental procedures, the cells were plated on glass coverslips and, at a near sub-confluence stage, were exposed to different doses of SMF (Teodori *et al.*, 2006).

Cell Treatment

The triggers used were:

- UV-B (312 nm) for 30 min, followed by 4h recovery,
- SMF 3000G, followed by 2h recovery.
- hyperthermia for 1h at 43°C, followed by 2-4 h recovery,
- hypothermia for 2h at 0 - 6°C, followed by 2-4 h at 37°C.

Transmission electron microscopy

Specimens were processed for transmission electron microscopy (TEM) according to conventional procedures (D'Emilio *et al.*, 2010). Briefly, pellets were washed and immediately fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) for 2h. After a gentle washing, a post-fixation was performed for 2h in 1% OsO₄ in the same buffer. Alcohol dehydration and araldite embedding were performed and thin sections, collected on nickel grids, and stained with uranyl acetate and lead citrate were analysed with a Philips CM10 electron microscope.

Scanning electron microscopy

After washing, specimens were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 1h. They were quickly washed in 0.15 M phosphate buffer. The samples were then washed and post-fixed with 1% OsO₄ in the same buffer for 1h. A progressive alcohol dehydration was performed, followed by specimen critical point drying. After mounting on conventional scanning electron microscopy (SEM) stubs by means of silver glue, specimens were gold-sputtered (Sestili *et al.*, 2006). Observations were carried out with a Philips 515 scanning electron microscope.

Results

UVB-Irradiation

UV-B- radiations induced apoptotic patterns are

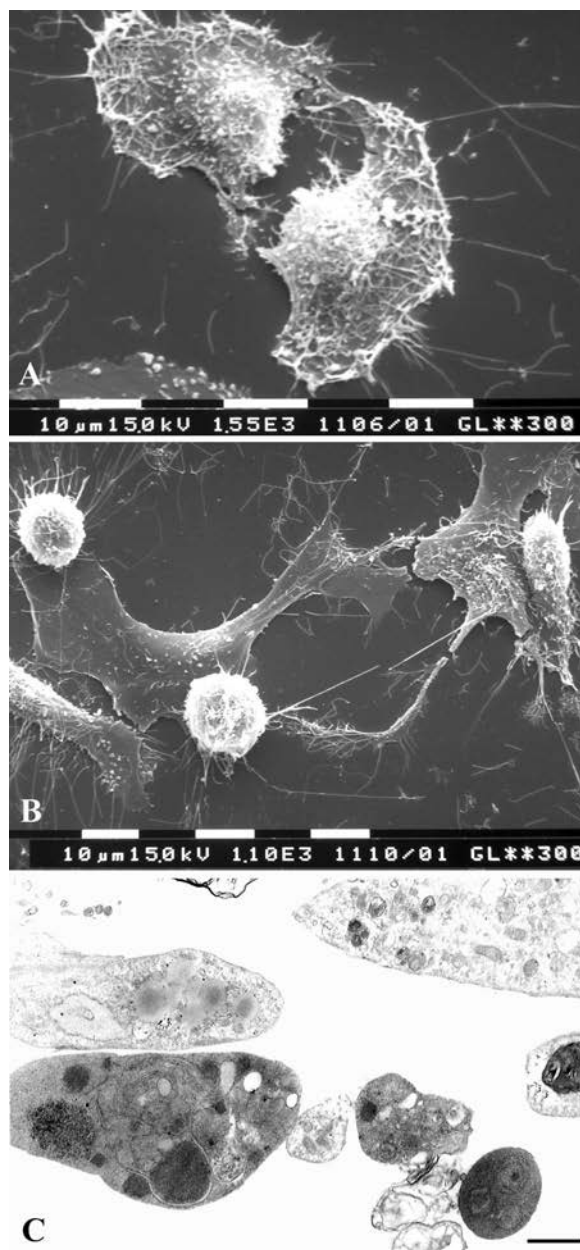


Figure 2. SEM (A, B) and TEM (C) of glioblastoma SMF-treated cells. Progressive cell detachment, loss of the long projections (A) and occasional spherical apoptotic cells appear (B). Micronuclei and vacuoles can be also revealed (C). A, B, C, Bar = 10 μm

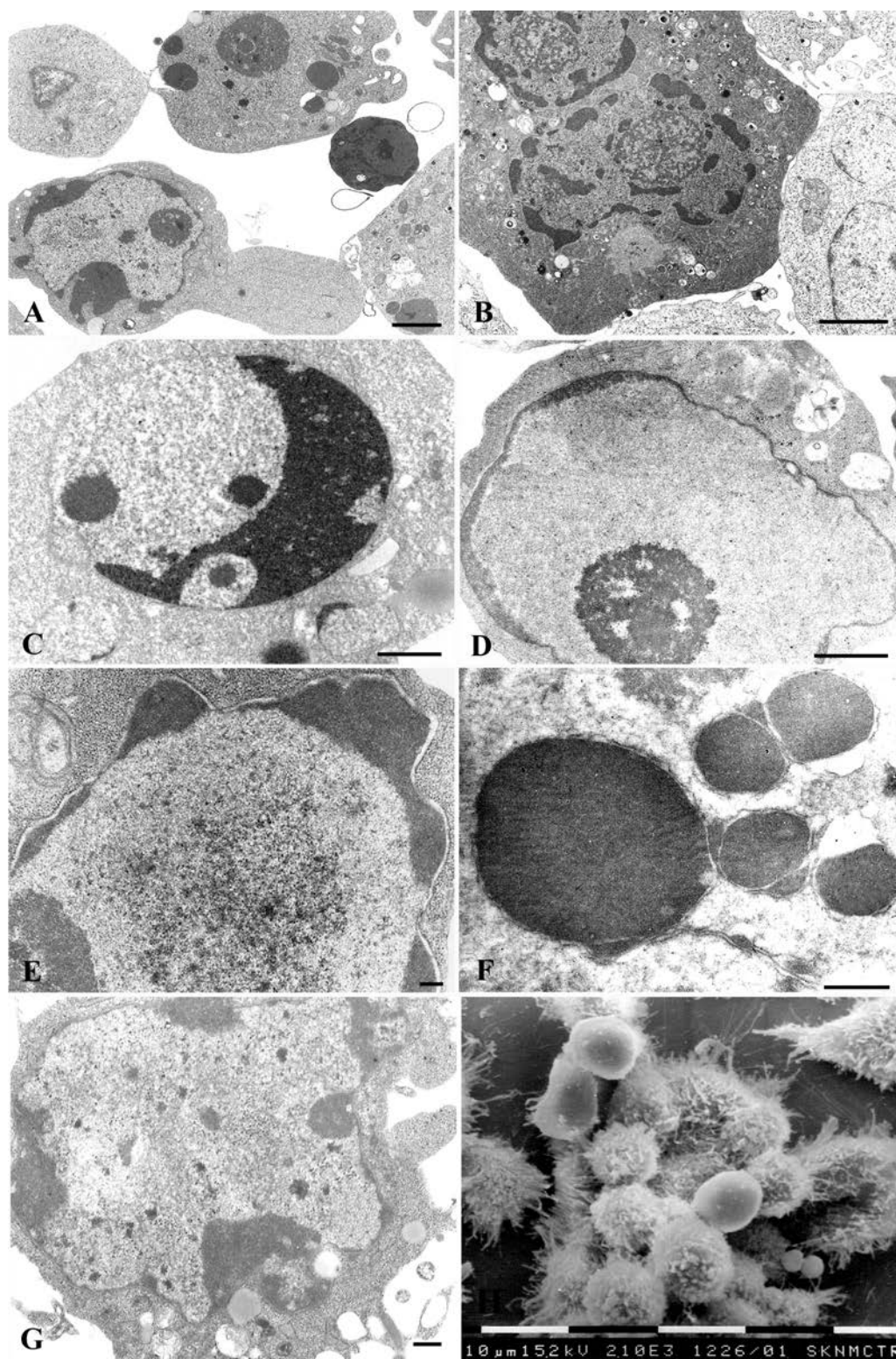


Figure 3. Hyperthermia induces nuclear changes in HL60 (A), U937 (B), MOLT-4 (C), K562 (D) and P815 (E, F) cells. Progressive chromatin condensation appears at TEM observation (A, B, C, D, E), as well as micronuclei (A, F). SK-N-MC cells show chromatin condensation (G) and progressive cell rounding with detachment from the substrate (H). A, B, C, D, E, F, G, H, Bar = 1 μ m

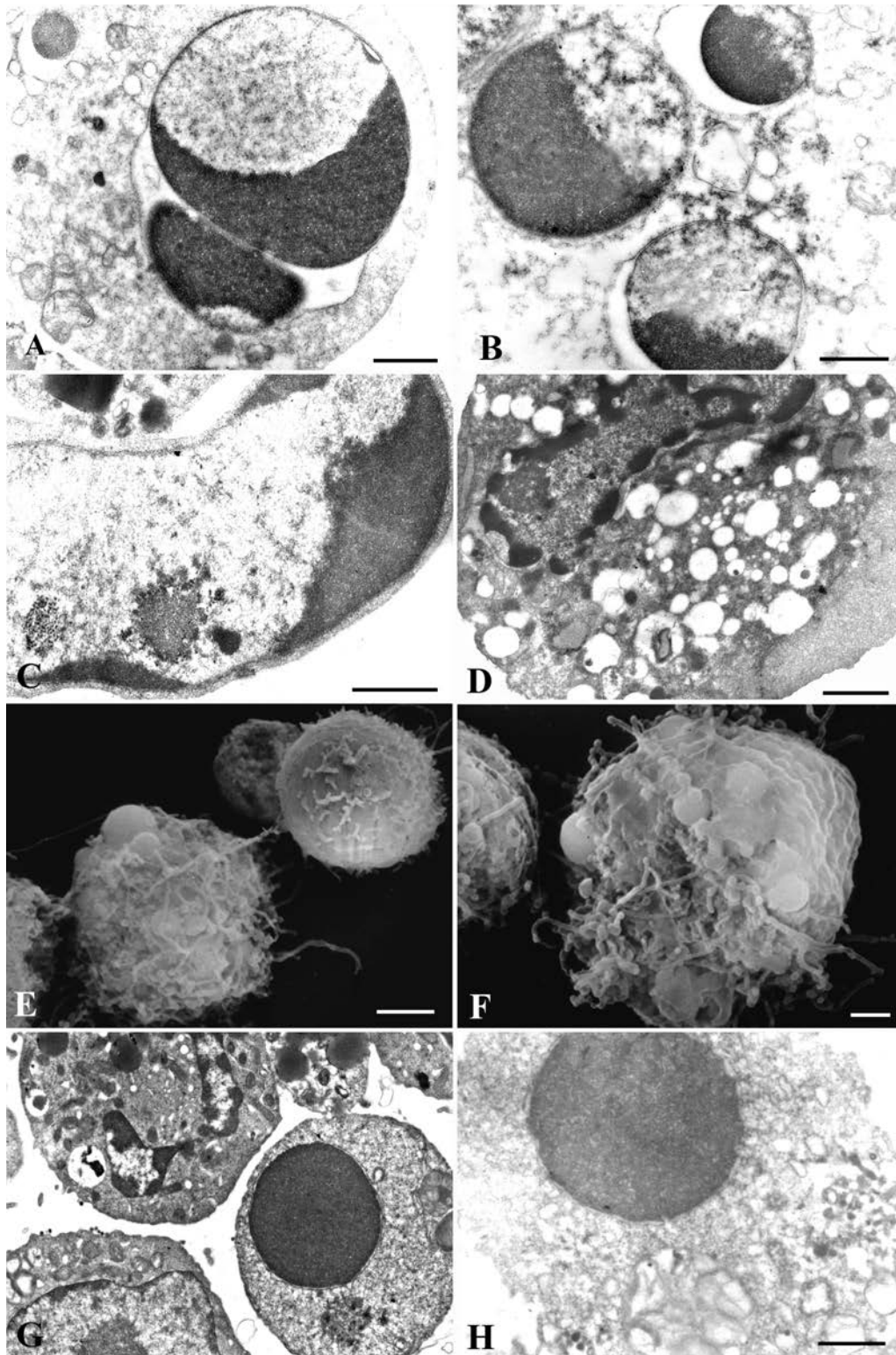


Figure 4. MOLT-4 (A, B), K562 (C, D), and P815 (E - H) cells after hypothermia treatment. MOLT-4 cells show micronuclei formation (A, B) and K562 evidence chromatin margination (C, D). Progressive cell rounding and detachment from the substrate can be revealed in P815 cells (E, F), which also show, at TEM observation, chromatin margination (G) and micronuclei (H). A, B, C, D, E, F, G, H, Bar = 1μm

shown in Figure 1. C2C12 murine myoblasts and myotubes showed morphological apoptotic changes. In myoblasts, a characteristic chromatin margination at the nuclear periphery frequently appeared (A). Even if cup-shaped masses, comparable to those of more classic apoptotic models, could not be found, the evidenced nuclear features, when analysed in detail, appeared to suggest apoptosis. An intriguing behaviour was revealed in myotubes, where apoptotic and normal nuclei appeared together within the same fiber (B). HL60 showed initial chromatin margination with traslocation of nuclear pores (C). Nuclear splitting and micronuclei occurred (D). Successively UVB-induced apoptosis in U937 (E, F) or in Molt-4 (G, H) cells showed chromatin condensation (E, G) and, particularly U937, cell shrinkage and blebs formation (F), with final nuclear splitting and micronuclei formation (F, H). Occasional autophagic vacuoles appeared in U937 treated cells (F). UVB also act by producing oxidative stress through an increased generation of Reactive Oxygen Species (ROS). Consequently, a mitochondrial involvement in UVB-induced apoptosis is certain and mitochondrial changes appear (F).

SMF treatment

We investigated the biological effects of SMF, in glioblastoma cells exposed to SMF 300 and 3000 G. Their effects are shown in Figure 2. SEM demonstrated a dose-dependent cell shape modification, with progressive cell detachment, loss of the characteristic projections (A) and occasional appearance of spherical apoptotic cells (B). At TEM observation, SMF modified cell shape changes also appeared with occasional presence of micronuclei and vacuoles (C).

Hyperthermia

Hyperthermia-induced apoptotic patterns are shown in figure 3. Hyperthermia (43°C for 1 hour) induced nuclear changes in HL60 (A), U937 (B), MOLT-4 (C), K562 (D) and P815 (E, F) cells. Progressive chromatin condensation appears at TEM analyses (A, B, C, D, E), generally followed by micronuclei formation (A, F). In SK-N-MC cells hyperthermia induces nuclear chromatin condensation (G) and a progressive cell rounding and detachment from the substrate, typical of apoptotic cell behaviour (H).

Hypothermia

Interestingly, apoptotic features characterize cell

injury after brief exposure to 0-6°C hypothermia, as shown in Figure 4. In MOLT-4 cells (A, B) micronuclei formation appeared. K562 cells, after hypothermic treatment, showed chromatin margination (C) with a diffuse cytoplasmic vacuolization (D), with occasional presence of autophagic vacuoles (D). Similarly, could induced a progressive cell rounding and blebbing in P815 cells (E), which showed, at TEM observation, chromatin margination (G) and micronuclear formation (H) too.

Conclusions

When compared to the chemical induction of apoptosis, relatively little is known about cell treatment by means of physical agents. UVB radiation, which represents a recent source of interest because of its role in skin aging and cancer, is a potent apoptotic inducer in hemopoietic tumor cell lines, where its mode of action is now relatively well known. Their application to other cell models should hopefully be carried out. Intriguingly, skeletal muscle cells, known to be resistant to apoptotic death, appear sensitive to UVB-induced apoptosis both as undifferentiated myoblasts, and in form of highly differentiated myotubes. The typical chromatin condensation with progressive compacting and formation of micronuclei, appear indeed at morphological investigation. High doses of static magnetic fields generate reproducible effects on targeted cells. We can suggest, however, that the principal cytotoxic damages resulting from magnetic field exposure, only occurs at the level of the plasma membrane and its surface specializations, while it rarely induces apoptosis. Hyperthermia has been revealed to be a powerful apoptotic trigger, widely utilized *in vitro*, where it induces the typical apoptotic features. Hypothermia, even if occasionally inducing apoptotic cell death, seems to stimulate, more diffusely, cell necrosis. Taken together, our data evidence a variable, but generally significant, apoptotic response to physical agents.

Acknowledgements

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Precipitation sequence in a QE22 magnesium alloy

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Summary

The hardening phase evolution in a QE22 Mg alloy is followed by differential scanning calorimetry, microhardness and transmission electron microscopy, after different thermal treatments. The decomposition of the supersaturated solid solution occurs via the formation of nanometric coherent structures (co-clusters or GP zones) followed by the co-precipitation of two metastable phases responsible for the peak ageing condition. The stable phase $(\text{Mg,Ag})_{12}\text{Nd}$ appears at the highest annealing times, giving rise to over-ageing and hardness reduction. TEM observations provide information on the crystallographic structure of the forming phases.

Key words: precipitation hardening, thermal treatments, differential scanning calorimetry, transmission electron microscopy.

Introduction

The increasing demand of weight reduction in the automotive and aeronautical industry has renewed the interest in the study of magnesium alloys, despite its relatively high production costs. Magnesium is a good potential element for many structural applications, although the ageing response of magnesium alloys is not as relevant as that of aluminium alloys, but the addition of particular solute atoms has proved to increase the tensile properties as well as the hardness of several Mg-based compositions (Polmear, 2006). In particular, it has been shown that the addition of 2-3 wt.% Ag in rare-earth-containing alloys markedly improves their age hardening response and hence their mechanical properties (Payne and Bailey, 1959-1960). This paper studies the phase transformations occurring in a Mg-Nd-Ag-Zr commercial alloy (commonly named QE22) when submitted to different thermal treatments. QE22 is part of a series of alloys with tensile properties comparable with the high-strength aluminium casting alloys and it has been used for a number of aeronautical applications (Kannan *et al.*, 2008; Luo and Pekguleryuz, 1994). In this paper a structural ana-

lysis of the precipitation sequence in a QE22 magnesium alloy is reported. Investigations were carried out by differential scanning calorimetry (DSC) analyses, microhardness tests and transmission electron microscopy (TEM) observations.

Materials and Methods

As-cast commercial QE22 bars with composition Mg-2.2Nd-2.1Ag-0.6Zr (wt%) were used. Slices about 1 mm thick were cut, from which small discs about 5 mm in diameter were obtained by punching. The discs were solution heat treated in a vertical furnace at 530°C for 6 hours, then quenched in water at room temperature. After this treatment they were stored at about -18°C in order to avoid phase transformations before performing annealing and analyses. The DSC measurements were carried out by a TA2010 calorimeter in a protective argon atmosphere. Pure Al with approximately the same weight as the sample (≈ 50 mg) was used as a reference. Thermal treatments were performed in an air-ventilated oven, at the highest temperature, or in a horizontal electrical furnace.

Microhardness tests were performed on the same samples used for calorimetric scans. Vickers hardness numbers were obtained with a load of 3 N by averaging at least five indentations.

TEM observations were performed at 200 kV with a Philips CM200 electron microscope equipped with a LaB₆ cathode. Samples were mechanically ground by emery papers, and an ultrasonic disc cutter (Gatan 601) was used to obtain discs with a diameter of 3 mm. The mechanical pre-thinning of a central area of each disc was performed by a dimple grinder (Gatan 656) while the final thinning was carried out in a precision ion polishing system (Gatan 691 PIPS) at 5 kV with the two guns at 4° of incidence.

Results and Discussion

The DSC trace of the solution treated and water quenched (AQ) sample at a scanning rate of 5 K·min⁻¹ is reported in Figure 1. As it is well known, the DSC technique is able to monitor phase transformations on scanning in temperature by revealing the specific heat variations due to atomic reorganization. At a first approximation, depletion of solute atoms from the out-of-equilibrium solid solution produces an exothermic peak of precipitation and vice versa for the endothermic signals.

In Figure 1, little can be said for the first part of the scan at low temperature, except for the formation of an endothermic signal D, positioned at ~200°C, following a region in which a broadened exothermic effect seems to be present. This implies that some aggregates, present just after

quenching or developing during the scan at low temperatures, dissolve near 200°C, giving rise to the signal D.

TEM observations of the AQ sample, reported in a previous work, show a fine precipitation of nanometric structures uniformly distributed inside the grains (Barucca *et al.*, 2009). Although this precipitation is clearly visible in bright field images, it does not produce any remarkable effect on the diffraction pattern, suggesting a complete coherency of the nanometric structures with Mg. It is most probable that such nanometric structures are co-clusters or Guinier-Preston zones (Starink and Wang, 2009; Lambri and Riehmman, 2005; Polmear, 2006).

Above 200°C, three exothermic signals P1, P2 and P3 appear in Figure 1. The origin of these effects can be investigated by heat treating the samples at temperatures suggested by the signal positions.

Actually, the scan of the AQ sample in Figure 1 shows (also taking into account the thermal drag due to the scanning rate) that the temperature value of 204 °C is positioned just after the pre-precipitates dissolution peak (signal D) and before any appreciable subsequent precipitation signal. Therefore, the precipitation effects responsible of the signals P1 and P2 can be studied by ageing the alloy around that temperature (204°C). Furthermore, in order to fully characterise the entire precipitation sequence of the alloy, two more temperature values at 150°C (before the signal D) and 275 °C (just after the peak P2) were considered for the ageing treatments (Figure 1).

The calorimetric traces of the samples treated at 204°C for various ageing times are reported in Figure 2. Here, the progressive disappearance of

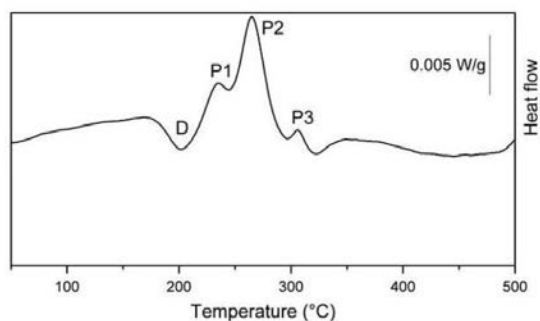


Figure 1. DSC trace for the solution treated and water quenched (AQ) sample. Scanning rate: 5 K min⁻¹.

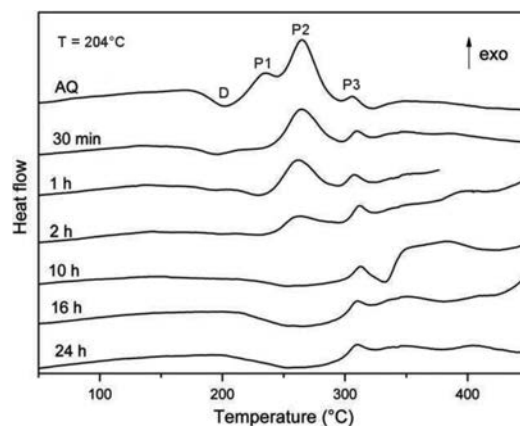


Figure 2. DSC traces after ageing at 204 °C for the labelled times. Scanning rate: 5 K min⁻¹.

the precipitation peaks P1 and P2, as well as the dissolution signal D at the increasing of the ageing time is clearly visible. In particular, it is worth to note that the sample aged for 10 hours at 204 °C (ageing treatment T6) shows only the exothermic signal P3 corresponding to a precipitation effect. This means that during the thermal treatment at 204 °C the phases responsible for the precipitation peaks P1 and P2 were formed while the phase formation or transformation linked to the P3 signal did not occur.

The alloy hardness evolution on ageing at 150 °C (triangles), 204 °C (squares) and 275 °C (dots) is reported in Figure 3. The error bar represents the standard deviation associated to each data point while the curves are only a guide for the eye.

The hardness evolution at 204 °C confirms that, after 10 hours at this temperature, the peak ageing condition is reached, after which over-ageing occurs, leading to a decrease in hardness.

TEM observations of the sample in the T6 condition, performed in the $\langle 001 \rangle_{Mg}$ zone axis orientation, reveal the presence of fine precipitates uniformly distributed inside the grains with dimension ranging from 10 to 50 nm, Figure 4a. The corresponding selected area diffraction (SAD) pattern of the sample is reported in figure 4b (upper part), together with the simulation (lower part) realized by the Crystal-KitX program (CrystalKitX software). Here, the most evident effect is the triangular structure located at $\sim 1/3\{-1-10\}_{Mg}$. This triangular arrangement of spots, as confirmed by

the simulation, can be ascribed to diffraction from a secondary phase and to double diffraction effects. TEM observations performed under different orientations allow evaluating the lattice parameters of this secondary phase and its orientation relationships with the matrix. In the following, this secondary phase is referred to as the χ phase. Results show that the χ phase is hexagonal with $a=0.286$ nm and $c=0.521$ nm, and it precipitates

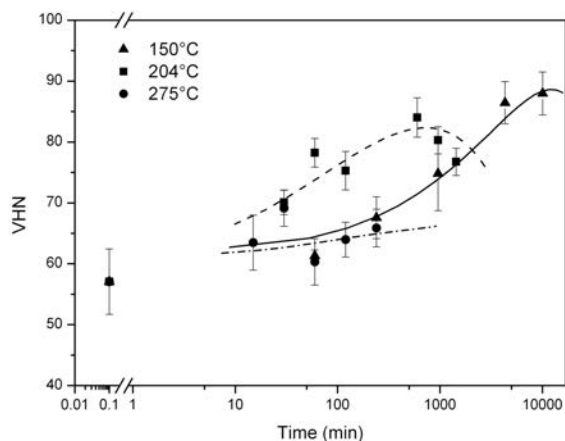


Figure 3. Microhardness evolutions on ageing at 150 °C (triangles), 204 °C (squares) and 275 °C (dots). The error bars are reported as the standard deviation of the measured indentations.

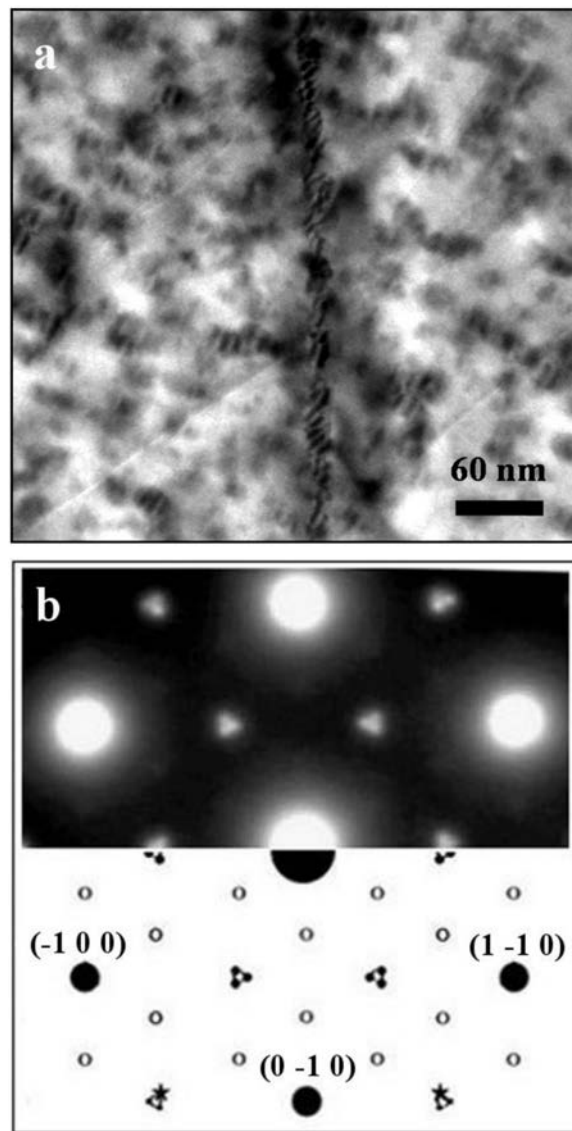


Figure 4. Sample aged 10 h at 204 °C (T6) observed in $\langle 001 \rangle_{Mg}$ zone axis orientation: (a) bright field, (b) SAD pattern (upper part) and SAD pattern simulation (lower part): large solid dots - Mg, open dots - γ phase, small solid dots - double diffraction, solid stars - χ phase.

according to the following orientation relationships with Mg:

$$\begin{aligned} (001)_{\chi} &\parallel (001)_{\text{Mg}} \\ [1-10]_{\chi} &\parallel [110]_{\text{Mg}} \end{aligned}$$

In the SAD pattern of Figure 4b (upper part), also faint extra spots located at $1/3\{1-10\}_{\text{Mg}}$ due to the metastable hexagonal γ phase are visible. SAD analyses performed under different crystal orientations suggest the following lattice parameters and orientation relationships of the γ precipitates with the Mg matrix:

$$\begin{aligned} a_{\gamma} &= 0.963 \text{ nm} \\ c_{\gamma} &= 1.035 \text{ nm} \end{aligned}$$

$$\begin{aligned} (001)_{\gamma} &\parallel (001)_{\text{Mg}} \\ [1-10]_{\gamma} &\parallel [110]_{\text{Mg}} \end{aligned}$$

The complete spot indexation obtained by the Crystal-KitX program considering both the χ and γ phases is reported in the lower part of Figure 4b (large solid dots - Mg, open dots - γ phase, small solid dots - double diffraction, solid stars - χ phase).

Hence, as a first consequence of the results obtained so far, one can conclude that the simultaneous presence of the χ and γ precipitates, linked to the P1 and P2 signals, is responsible for the maximum age hardening of the QE22 alloy.

As already discussed above, for a complete description of the precipitation sequence outlined in Figure 1, following the position in temperature of the calorimetric signals, two other ageing treatments were studied, at 150°C and 275°C.

The calorimetric traces of the samples aged at 150°C for different times show the permanence of the P2 signal even after 16 hours (960 minutes) of ageing and the persistence of a significant dissolution D signal up to the higher ageing times.

A bright field image taken in $\langle 100 \rangle_{\text{Mg}}$ orientation of the sample aged 16 hours at 150 °C is reported in Figure 5. Nanometric precipitates uniformly distributed inside the grains are clearly visible. The corresponding $\langle 100 \rangle_{\text{Mg}}$ zone axis SAD pattern (not shown here) presents extra spots only due to magnesium oxide (MgO), suggesting the absence of the γ phase, that is generally easily visible in this orientation.

On the contrary, the SAD pattern taken in $\langle 001 \rangle_{\text{Mg}}$ orientation shows well defined extra spots at $\sim 1/3\{-1-10\}_{\text{Mg}}$ compatible with the presence of the χ phase, Figure 6. The ring-shaped effects are due to magnesium oxide (MgO).

In conclusion, the γ phase has not yet formed after 16 hours at 150°C, and this explains the permanence of the P2 signal in the DSC traces. The hardening increases at this ageing temperature are due to the χ phase as well as to the formation of nano-sized coherent aggregates.

The calorimetric traces for the samples treated at

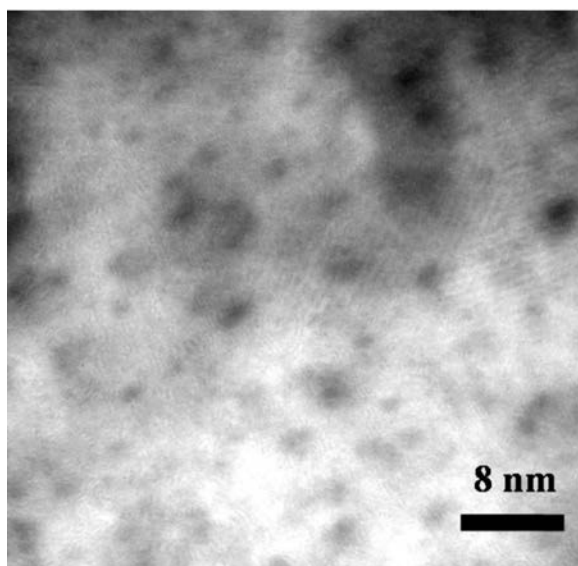


Figure 5. Bright field image of the sample aged 16 hours at 150 °C in $\langle 100 \rangle_{\text{Mg}}$ orientation.

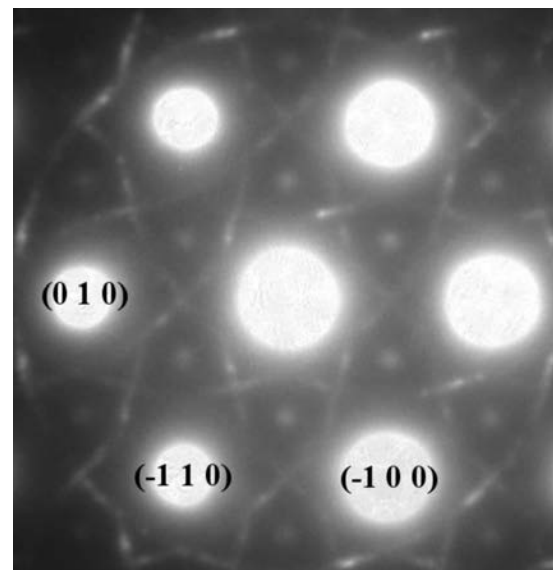


Figure 6. SAD pattern of the sample aged 16 hours at 150°C taken in $\langle 001 \rangle_{\text{Mg}}$ zone axis.

275°C show that all the precipitates are already formed just after 30 minutes of ageing. TEM observations performed on samples aged at 275°C allowed to reveal the nature of the stable phase.

Figure 7 reports the main TEM results obtained for the sample aged 30 minutes at 275°C and observed in $\langle 001 \rangle_{\text{Mg}}$ zone axis. In this sample,

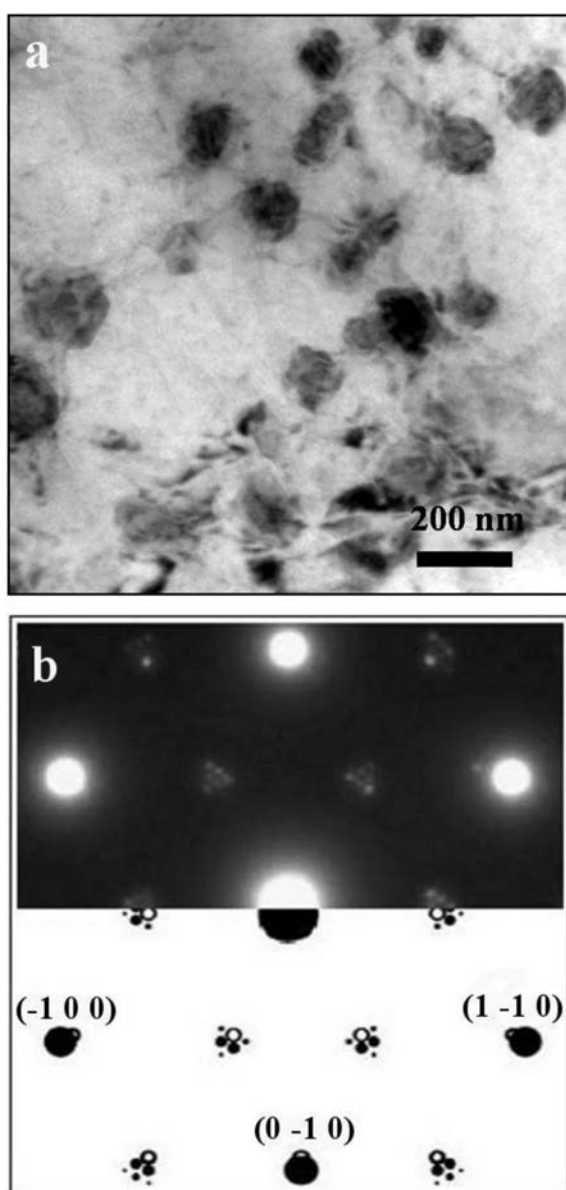


Figure 7. Sample aged 30 min at 275°C observed in $\langle 001 \rangle_{\text{Mg}}$ zone axis: (a) bright field image, (b) SAD pattern (upper part) and SAD pattern simulation (lower part): large solid dots - Mg, open dots - $(\text{Mg, Ag})_{12}\text{Nd}$ phase, small solid dots - double diffraction.

coarse precipitates preferentially located on dislocations and grain boundaries with dimension ranging from 30 to 170 nm, are present, Figure 7a.

The SAD pattern reported in Figure 7b (upper part) exhibits extra spots arranged in a triangular structure located at $\sim 1/3\{-1-10\}_{\text{Mg}}$. Although the general arrangement and the location of the spots is quite similar to those observed in the T6 sample (Figure 4b) and attributed to the χ phase, a closer look at the diffraction pattern of Figure 7b (upper part) reveals that the spots are now well separated, and their number has increased with respect to those observable in figure 4b.

The pattern simulation reported in figure 7b (lower part) allowed to attribute these extra spots to the stable $(\text{Mg, Ag})_{12}\text{Nd}$ phase and to double diffraction effects (large solid dots - Mg, open dots - $(\text{Mg, Ag})_{12}\text{Nd}$ phase, small solid dots - double diffraction).

Measurements carried out on the different SAD patterns, within experimental uncertainty, provide for the stable $(\text{Mg, Ag})_{12}\text{Nd}$ phase a tetragonal structure with $a=1.03$ nm and $c=0.59$ nm, and the following orientation relationships with Mg:

$$\begin{aligned} (100)_{(\text{Mg, Ag})_{12}\text{Nd}} &\parallel (001)_{\text{Mg}} \\ [0-11]_{(\text{Mg, Ag})_{12}\text{Nd}} &\parallel [110]_{\text{Mg}} \end{aligned}$$

All the SAD patterns taken in any other orientation are consistent with the presence of the stable $(\text{Mg, Ag})_{12}\text{Nd}$ phase. Hence, one can conclude that the P3 signal visible in the calorimetric traces of figure 1 can be attributed to the formation of the stable $(\text{Mg, Ag})_{12}\text{Nd}$ phase. Moreover, also the hardness trend at 275°C (Figure 3, dots and dash-dotted line), showing only a small increase during ageing, confirms the formation of the stable phase.

Conclusions

The precipitation sequence responsible for the age hardening of the QE22 alloy, was studied by DSC and TEM measurements. The solution heat treated samples were submitted to thermal treatments at 150 °C, 204 °C and 275 °C for different ageing times and the results can be summarized as follows:

- the decomposition of the supersaturated solid solution occurs via the formation of nanosized coherent structures (co-clusters or GP zones)

- followed by the co-precipitation of two metastable hardening phases named χ and γ ;
- at 150°C, the hardening increase is due to the permanence of the zones and to the precipitation of a metastable hexagonal phase, here named χ ;
 - at the T6 (16 hours at 204°C) peak ageing condition a co-precipitation of χ and γ occurs;
 - at 275 °C, only the stable $(\text{Mg,Ag})_{12}\text{Nd}$ phase is present, even after the shortest ageing times.
- The diffraction patterns can be correctly indexed and simulated when assuming the following structures:
- an hexagonal χ phase with $a_\chi=0.286$ nm and $c_\chi=0.521$ nm and the following orientation relationships with the matrix: $(001)_\chi \parallel (001)_{\text{Mg}}$, $[1-10]_\chi \parallel [110]_{\text{Mg}}$.
 - an hexagonal γ phase with $a_\gamma=0.963$ nm and $c_\gamma=1.035$ nm and the following orientation relationships with the matrix: $(001)_\gamma \parallel (001)_{\text{Mg}}$, $[100]_\gamma \parallel [100]_{\text{Mg}}$.
 - a tetragonal $(\text{Mg,Ag})_{12}\text{Nd}$ stable phase with $a=1.03$ nm and $c=0.59$ nm and the following orientation relationships with the matrix: $(100)_{(\text{Mg,Ag})_{12}\text{Nd}} \parallel (001)_{\text{Mg}}$, $[0-11]_{(\text{Mg,Ag})_{12}\text{Nd}} \parallel [110]_{\text{Mg}}$.

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In vitro ageing of myotubes derived from myoblasts of patients affected by myotonic dystrophy type 2: ultrastructural evidence

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Summary

Myotonic dystrophy type 2 (DM2) is a dominantly inherited autosomal disease with multi-systemic clinical features and is caused by expansion of a CCTG tetranucleotide repeat in the first intron of the zinc finger protein 9 (ZNF9) gene in 3q21. The expanded-CCUG-containing transcripts are retained in cell nuclear domains (called foci) which specifically sequester some splicing factors, thus causing a general alteration of the pre-mRNA post-transcriptional pathway that is likely responsible for the multifactorial phenotype of DM2 patients. However, at the skeletal muscle level, there is still no mechanistic explanation for the muscle weakness and atrophy of DM2 patients. It has been noted that in DM2 patients skeletal muscle regeneration is decreased, suggesting an impaired responsiveness of satellite cells to regeneration stimuli as much as it occurs in ageing muscles. In order to investigate the differentiation potential of senescing DM2 myoblasts and the development of the derived myotubes, we developed a model system of cell ageing *in vitro*: by this approach, the structural features of myotubes derived from DM2 myoblasts grown in culture for increasing times have been investigated by fluorescence and transmission electron microscopy. Apparent alterations of several cytoplasmic features have been observed in the myotubes derived from myoblasts at higher passages. This strongly argues in favour of the involvement of satellite cell senescence in the reduced regenerative potential of dystrophic muscles.

Key words: myotonic dystrophy type 2-DM2, myoblast, myotube, cell senescence, fluorescence microscopy, electron microscopy.

Introduction

Myotonic dystrophy type 2 (DM2) is a dominantly inherited autosomal disease with multi-systemic clinical features and is caused by the expansion of a CCTG tetranucleotide repeat in the first intron of the zinc finger protein 9 (ZNF9) gene in 3q21 (Liquori *et al.*, 2001). The expanded-CCUG-containing transcripts are retained in the cell nucleus and accumulate in the form of focal aggregates (Liquori *et al.*, 2001): these nuclear foci of mutant mRNA specifically sequester essential splicing factors such as muscleblind-like (MBNL) proteins (Fardaei *et al.*, 2002, Cardani *et al.*, 2006, Pascual *et al.*, 2006), snRNPs and hnRNPs (Perdoni *et al.*, 2009a,b), leading to nuclear depletion and loss of

function of these regulators (Mankodi *et al.*, 2001). This strongly supports the hypothesis that a general alteration of the pre-mRNA post-transcriptional pathway could be at the basis of the multifactorial phenotype of DM2 patients (Wheeler and Thornton, 2004; Ranum and Cooper, 2006).

However, at the skeletal muscle level, there is still no mechanistic explanation for the muscle weakness and atrophy of DM2 patients, or for the histopathological features of this disease which include fibre atrophy-hypertrophy, increased number of centrally located nuclei, and presence of fibres with nuclear clumps. It has been noted that in DM2 patients skeletal muscle regeneration is decreased in response to the ongoing muscle loss and dystrophy (Harper *et al.*, 2001); it is possible

that this poor repair response may result from impaired myogenesis in the adult DM2 muscle. Interestingly, DM muscle shares apparent similarities with the ageing muscle where the progressive muscle weakness and atrophy is accompanied by a slower regenerative capability (Schultz and Lipton, 1982; Machida and Narusawa, 2006; Verdijk *et al.*, 2007).

In skeletal muscles, the myoblasts precursor cells called satellite cells provide the potential for both pre- and post-natal growth, as well as for muscle regeneration following injury (Moss and Leblond, 1971). It has been conclusively demonstrated that myoblast precursor cells, which are quiescent in normal muscles, become activated following injury: they then proliferate and fuse into myotubes which finally differentiate and become muscle fibres (Bischoff and Heintz, 1994; Cooper *et al.*, 1999). It is likely that a failure in satellite cell activation or a change in the behaviour of the activated satellite cells could contribute to the development of the dystrophic phenotype of DM2 skeletal muscles.

In vitro myoblast cultures derived from human satellite cells provide a suitable and unique model for studying DM2 muscular precursor cells, and can be used to elucidate the molecular and cellular mechanisms involved in the pathogenesis of this disease (Mankodi *et al.*, 2003; Cardani *et al.*, 2004).

The aim of this study was to investigate, by fluorescence and transmission electron microscopy, the structural features of differentiating myotubes derived from DM2 myoblasts grown in culture for increasing lengths of time, in order to investigate the differentiation potential of senescing myoblasts and the development of the derived myotubes.

Materials and Methods

Sample collection and cell culture

The biopsies used in this study were taken, under sterile conditions, from the biceps brachii of two DM2 patients, after informed consent. The histological diagnosis was performed on serial sections processed for routine histological or histochemical staining, based on the clinical criteria set by the International Consortium for Myotonic Dystrophies (Moxley *et al.*, 2002). The biopsies from donors were

trimmed of blood vessels, fat and connective tissues, and rinsed in phosphate-buffered saline (PBS); satellite cells were isolated as reported in Cardani *et al.* (2009) and placed in HAM's F10 medium (Sigma-Aldrich, Buchs, Switzerland) supplemented with 15% fetal bovine serum (Gibco, Invitrogen, Milan, Italy), 0.5 mg/mL bovine serum albumin (BSA), 0.5 mg/mL fetuin, 0.39 µg/mL dexamethasone, 10 ng/mL epidermal growth factor, 0.05 mg/mL insulin, 3 mg/mL glucose, 100 U/mL penicillin and 100 µg/mL streptomycin (all these reagents were from Sigma-Aldrich). The myoblasts obtained by this procedure were propagated in plastic flasks at 37°C in a humidified 95% air / 5% CO₂ atmosphere.

Proliferating myoblasts at the 9th (M9) and 12th passage (M12) in culture were used (their S-phase index being about 10% and 3%, as preliminarily estimated by BrdU incorporation experiments, not shown). M9 and M12 myoblasts were plated onto glass coverslips, and allowed to grow until they were 80% confluent. To initiate differentiation, the proliferative medium was replaced with differentiation medium consisting of DMEM supplemented with 7% FBS, containing 100 U/mL penicillin and 100 µg/mL streptomycin. Myoblasts were allowed to fuse and differentiate into myotubes for 8 (T8), 12 (T12), and 22 (T22) days.

Light microscopy

For fluorescence cytochemistry, myotube cultures were fixed with 4% formaldehyde in PBS for 15 min at 4°C, washed in PBS at room temperature, and incubated with either an Alexa 488-conjugated phalloidin (Molecular Probes, Invitrogen, Milan, Italy) diluted 1:40, or a mouse monoclonal antibody recognizing fast-myosin (Sigma-Aldrich), diluted 1:400, finally revealed with an Alexa 594-conjugated secondary anti-mouse IgG (Molecular Probes). All these reagents were diluted in PBS containing 0.1% BSA and 0.05% Tween-20 for 60 minutes at room temperature. After washing in PBS, cells were counterstained for DNA with Hoechst 33258 (Sigma-Aldrich; 1 µg/mL for 5 min), and mounted in a drop of Mowiol (Calbiochem, Milan, Italy).

For fluorescence microscopy, an Olympus BX51 microscope equipped with a 100W mercury lamp was used, under the following conditions: 330-385 nm excitation (excf) filter, 400 nm dichroic mirror (dm), and 420 nm long-pass filter, for Hoechst 33258; 450-480 nm excf filter, 500 nm dm, and 515-550 nm band-pass filter for Alexa 488; 550 nm excf filter, 590 nm dm, and 620 nm long-pass filter for Alexa 594.

Images were recorded with a Camedia 5050 digital camera system, and stored on a PC by the Olympus software, for processing and printing.

A cytochemical assessment of mitochondrial activity was also performed by vital staining with 2 μ M JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide; Molecular Probes, Invitrogen, Italy) for 15 min at 37°C in a 5% CO₂ atmosphere. When administered to living cells, JC-1 accumulates in the mitochondria where it emits either red or green fluorescence, depending on the mitochondrial membrane potential, the red signal indicating polarized mitochondria, and the green signal the depolarized ones (Reers *et al.*, 1991); therefore, the shift from red to green fluorescence is considered a reliable indication of a drop in the mitochondrial membrane potential. JC-1 positivity was visualized under the fluorescence microscope using a 450-480 nm excf filter, 500 nm dm, and 520 nm long-pass filter.

Transmission electron microscopy

T8, T12 and T22 myotubes were fixed with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M Sörensen phosphate buffer at 4°C for 1 h, washed, post-fixed with 1% OsO₄ at room temperature for 1 h, dehydrated with acetone and embedded in Epon: gelatine capsules were filled with the resin and placed up-side down onto the coverslips; after polymerization, the glass coverslips were detached by immersion in liquid nitrogen. Ultrathin sections were stained with uranyl acetate and lead citrate, and finally observed in a Philips Morgagni TEM operating at 80kV and equipped with a Megaview II camera for digital image acquisition.

Results

Transmission electron micrographs of myotubes derived from M9 myoblasts at the differentiation steps T8, T12 and T22 are shown in Figure 1. In the thin sections, T8 myotubes from M9 myoblasts showed elongated shapes, with longitudinally arranged nuclei (Figure 1a) (about 25% of the myotubes in the cultures showed more than 5 nuclei); in the cytoplasm, several bundles of longitudinally arranged myofibrils mainly occurred at the cell periphery, and frequently showed sarcomere-like figures (Figure 1c), consistent with the evidence after immunolabeling for fast-myosin

(Figure 2a). Numerous elongated mitochondria with lamellar cristae and some osmiophilic granules in their matrix were either orderly arranged between myofibril bundles or clustered around the nuclei (Figure 1b). Large amounts of ribosomes were present, mostly free in the cytoplasm but also in association with the cisternae of the endoplasmic reticulum. Well developed Golgi complexes often occurred close to the cell nuclei. A well developed smooth endoplasmic reticulum was ubiquitously distributed, mostly in the form of small round vesicles. Many lysosomes and residual bodies with different size and content were also observed. The cell nuclei were generally elongated, with low amounts of condensed chromatin and a single reticular nucleolus (Figure 1a).

Some ultrastructural features of T12 myotubes from M9 myoblasts were different from those observed in the thin sections of T8 myotubes. In detail: the myofibril bundles appeared less numerous and thinner, although they still showed sarcomere-like arrangements (Figure 1d); the mitochondria exhibited well developed cristae and many osmiophilic granules (Figure 1d); the residual bodies markedly increased in number, and roundish cytoplasmic areas appeared to be filled with a finely granular electron-dense material (Figure 1e). Similarly to T8, cell nuclei were lined along the longitudinal axis of the myotubes (not shown).

In T22 myotubes from M9 myoblasts, thin myofibril bundles were present, which still showed some sarcomere-like arrangements but were more irregularly arranged (Figure 1f,g and Figure 2b): instead of running parallel, they could be convergent and sometimes even perpendicularly oriented (Figure 2c). Mitochondria were smaller and contained a few cristae (Figure 1g). The smooth endoplasmic reticulum and the heterogeneous residual bodies became prominent, and large cytoplasmic areas were filled with electron-dense fine granular material. Finally, in the majority of thin sections, only one nucleus was observed in the cytoplasm, with the same ultrastructural features as in T8 and T12 myotubes (not shown). This likely relates with the increasing length of the myotubes, since the mean number of nuclei per myotube did not change significantly from T8 to T22 (3.5 and 3.9 nuclei per myotube, respectively).

Figure 3 shows myotubes derived from M12 myoblasts at the differentiation steps T8, T12 and T22. In the thin sections, T8 myotubes from M12 myo-

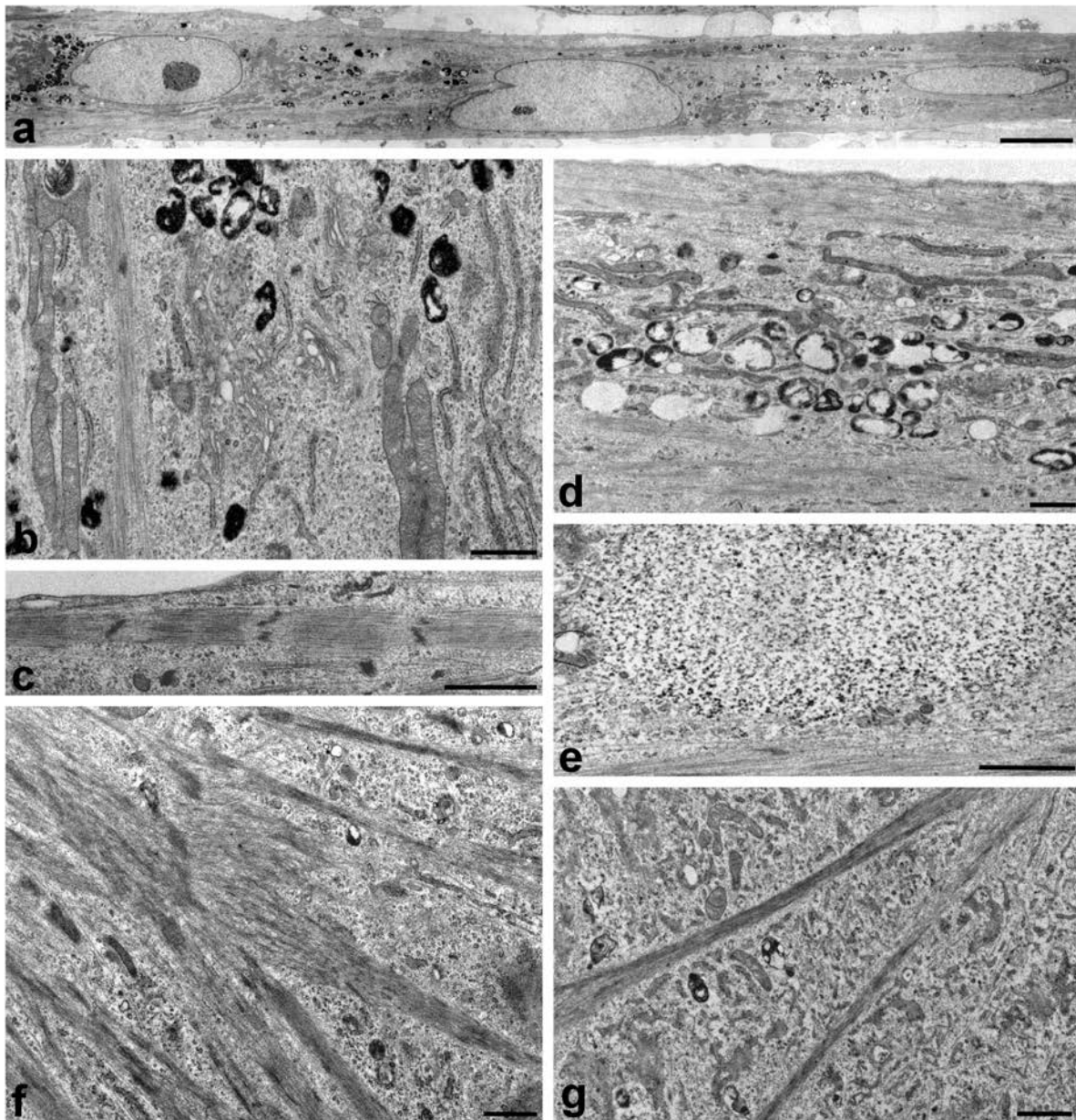


Figure 1. Electron micrographs of myotubes derived by M9 myoblasts at the differentiation step T8 (a-c), T12 (d,e) and T22 (f,g). (a) Myotube with three aligned nuclei. (b) Large amounts of free ribosomes, RER, well developed Golgi complexes, elongated mitochondria with lamellar cristae, and residual bodies occur in the myotube cytoplasm. (c) Detail of bundles of longitudinally arranged myofibrils showing sarcomeric-like arrangements. (d) In T12 myotubes the myofibril bundles still show sarcomeric-like arrangements, mitochondria contain many osmiophilic granules and residual bodies are prominent. (e) Cytoplasmic area filled with fine electron-dense granular material. (f,g) In T22 myotube the myofibril bundles are irregularly arranged and mitochondria are small with a few cristae; free ribosomes, rough and smooth endoplasmic reticulum are abundant. Bars: (a) 5 μm , (b-g) 1 μm .

blasts were ovoid in shape, with one to three roundish or irregularly shaped nuclei often lined transversally to the longitudinal axis of the cell (Figure 3a). In their cytoplasm, areas containing the usual organelles were found close to wide, electron-lucent regions containing heterogeneous vacuoles (Figure 3b). Myofibril bundles were scarce in number and very thin, and sarcomere-like arrangements were rarely observed (Figure 3c); moreover, they seldom had a regularly longitudinal orientation. Small elongated mitochondria with a few cristae were mostly distributed as clusters (Figure 3c). Large amounts of free ribosomes, many rough endoplasmic reticulum cisternae, and well developed Golgi complexes were present. Smooth endoplasmic reticulum, lysosomes and heterogeneous vacuoles were abundantly and ubiquitously distributed. The cell nuclei generally contained scarce condensed chromatin and one reticular nucleolus.

T12 and T22 myotubes from M12 myoblasts were characterised by more numerous large electron-lucent cytoplasmic areas containing heterogeneous vacuoles, and by the scarcity of myofibrils arranged in bundles (Figure 3e). Some mitochondria showed the usual morphology, whereas other appeared to be swollen and contained hardly recognizable cristae (Figure 3d). After JC-1 staining, most of the mitochondria predominantly emitted red fluorescence (not shown), thus indicating that they were still functioning and preserved their intermembrane potential. Free ribosomes, rough endoplasmic reticulum and Golgi complexes were restricted only to limited cytoplasmic areas (Figure 3d), whereas the smooth endoplasmic reticulum and the heterogeneous vacuoles were prominent throughout the cytoplasm. The cell nuclei generally showed scarce condensed chromatin and one reticular nucleolus.

Conclusions

Taken together, our observations demonstrate that the myotubes derived from DM2 myoblasts at the earlier culture passage (M9) underwent differentiation and showed the structural features already described for myotubes from normal myoblasts (Curci *et al.*, 2008; Bigot *et al.*, 2008). During their ageing in culture, these myotubes exhibited a progressive disorganization of the cytoskeletal apparatus and, to a lesser extent, a decrease in

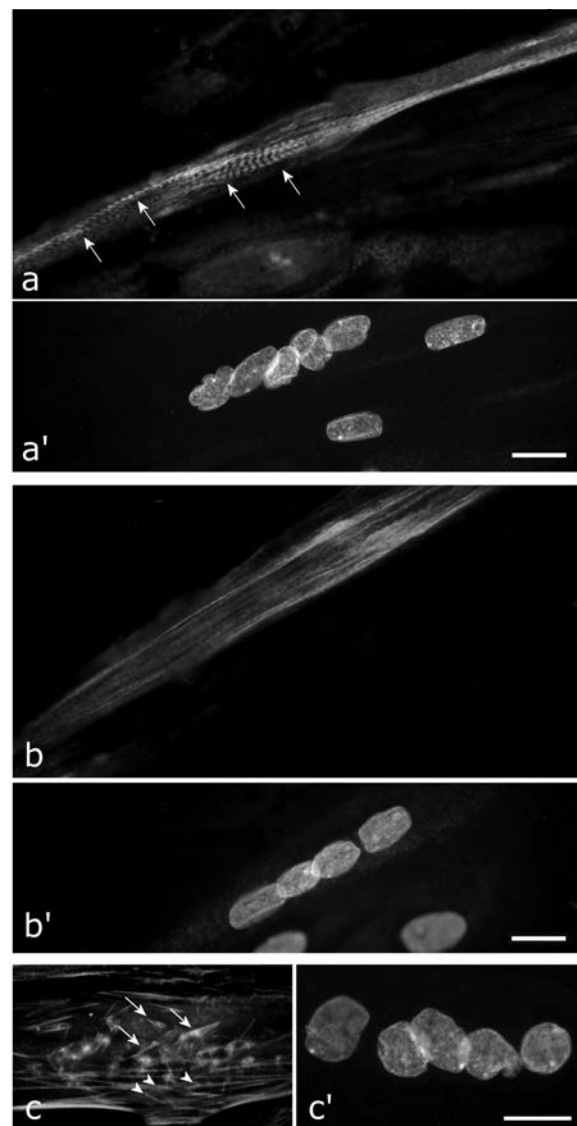


Figure 2. (a) A T8 myotube from M9 myoblasts is shown, after immunolabeling for fast-myosin: the arrows point to bundles of longitudinally arranged myofibrils; nuclear DNA was stained with Hoechst 33258 (a'). (b) A T22 myotube from M9 myoblasts after immunolabeling for fast-myosin and counterstaining of DNA with Hoechst 33258 (b'): myofibril organization is no longer visible. (c) Detail of a T22 myotube from M9 myoblasts after labeling of F-actin with phalloidin: actin bundles are irregularly arranged (arrows), and sometimes become convergent and perpendicularly oriented (arrowheads); nuclear DNA was stained with Hoechst 33258 (c'). Bars: 20 μ m

mitochondrial functions, as suggested by the loss of cristae and the concomitant accumulation of osmiophilic granules in their matrix (Jacob *et al.*, 1994; Malatesta *et al.*, 2001). Conversely, the proteosynthetic apparatus did not show massive morpho-functional alterations. Smooth vesicles and heterogeneous vacuoles markedly increased as it was observed to occur during cell senescence, in parallel with progressive malfunctioning of the degradation systems (Jameson, 2004).

As reported by Bigot *et al.* (2008), normal human myoblasts maintain their capability to fuse and differentiate after many passages in culture, when they reach replicative ageing and stop dividing: young myoblasts were found to form very long and branched myotubes, about 90% of the myonu-

clei being contained in myotubes with more than 50 nuclei; senescent normal myoblasts formed much shorter myotubes, and less than 40% of the myonuclei were found in myotubes with 25 nuclei or more. This suggests the occurrence of a defect in the differentiation and/or fusion capabilities of normal myoblasts as long as they become senescent (Bigot *et al.*, 2008). Our results are consistent with these observations, but also indicates that even the proliferating M9 myoblasts from DM2 patients form much shorter myotubes with a relatively low number of nuclei. In addition, already at a relatively early differentiation step (T12) the myotubes derived from M12 myoblasts showed features suggestive of degenerating myotubes: i.e., cytoskeleton disorganization, small mito-

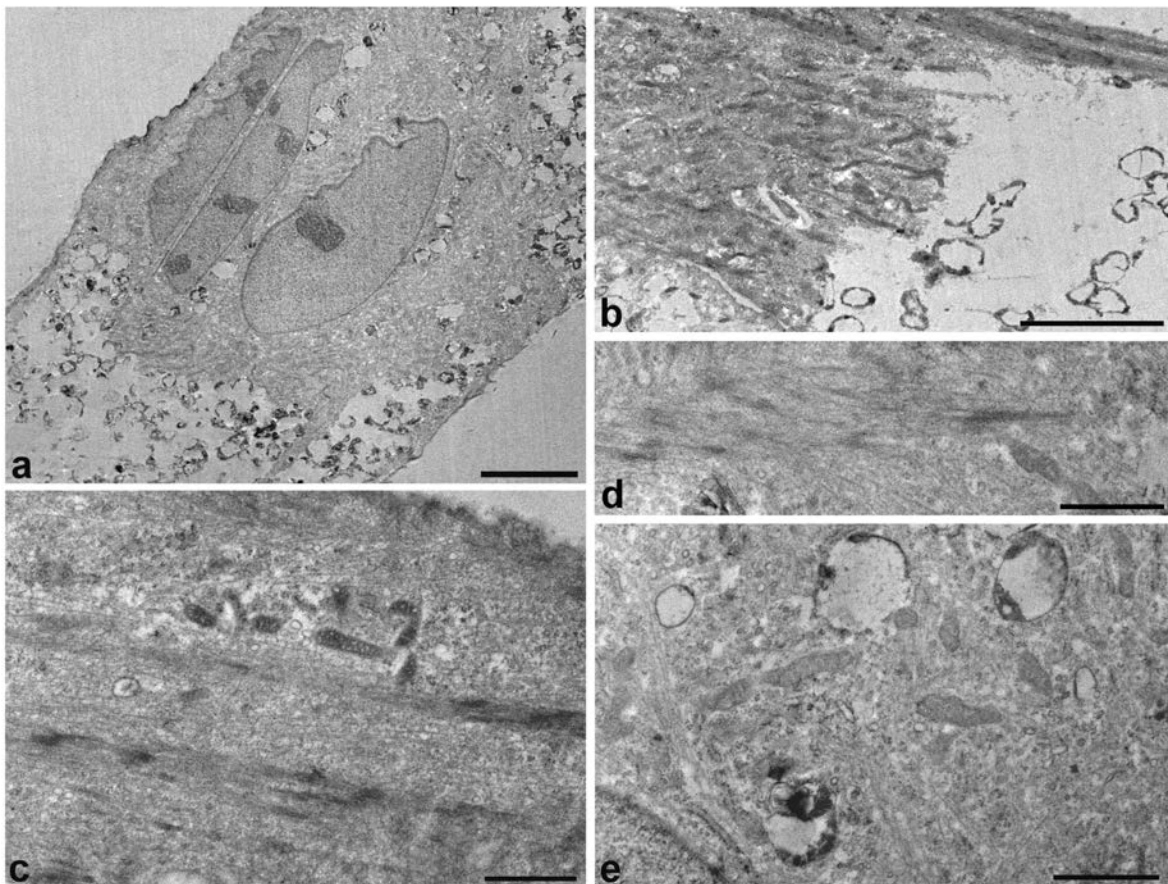


Figure 3. Electron micrographs of myotubes derived by M12 myoblasts at the differentiation step T8 (a-c), T12 (d) and T22 (e). (a) Myotube with 3 transversally aligned nuclei. (b) Cytoplasmic areas containing the usual organelles are close to wide, electron-lucent regions containing heterogeneous vacuoles. (c) Myofibril bundles are thin and show a few sarcomeric-like arrangements; small mitochondria are distributed as clusters. (d) In T12 myotubes the myofibrils are disorganized. (e) In T22 myotubes mitochondria contain hardly recognizable cristae, smooth vesicles are numerous, and free ribosomes and RER are abundant. Bars: (a,b) 5 µm, (c-e) 1 µm.

chondria with a few recognizable cristae, and large amounts of heterogeneous vacuoles. At the subsequent steps in differentiation medium, cytoplasmic degeneration became more apparent; however, it is worth noting that the proteosynthetic apparatus as well as the cell nuclei did not seem to be severely affected even at the latest differentiation times analyzed; even the mitochondrial population was only partially damaged at T22, and a fraction of mitochondria preserved their normal intermembrane potential, as demonstrated by JC1 staining. Interestingly, no structural alteration typical of apoptotic cell death (Burattini *et al.*, 2004; D'Emilio *et al.*, 2010) was found in our experimental conditions, contrary to what observed in 15-day differentiated DM1 myotubes (Loro *et al.*, 2010). This difference could be related to the more severe consequences of the nuclear sequestration of defective mRNA in the nuclei of DM1 compared to DM2 cells.

In conclusion, our study confirms and extends previous data showing that DM2 myoblasts in culture can be induced to differentiate into myotubes (Cardani *et al.*, 2009; Pelletier *et al.*, 2009), but their differentiation potential markedly decreases in parallel with their senescence *in vitro*. The structural alterations early observed in the myotubes from senescing myoblasts suggest that also *in vivo* the differentiation potential of satellite cells in DM2 patients could be low, thus compromising their capability to repair dystrophic muscles.

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