microscopie

Anno VII - n. 1 (13) - Marzo 2010

Premio SISM 2010
Attività SISM 2010
Vincitori del concorso "In copertina su Microscopie"
IMC17

Società Italiana
Scienze Microscopiche

www.sism.it

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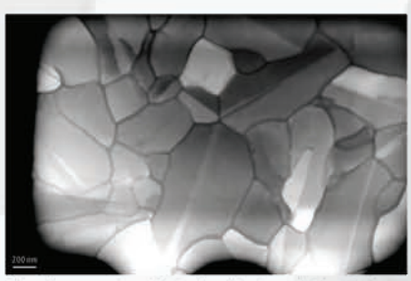
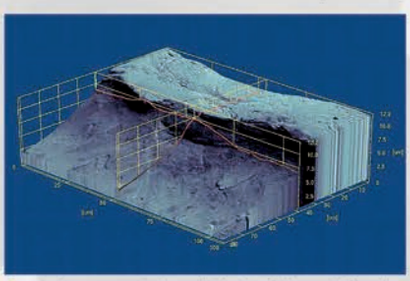
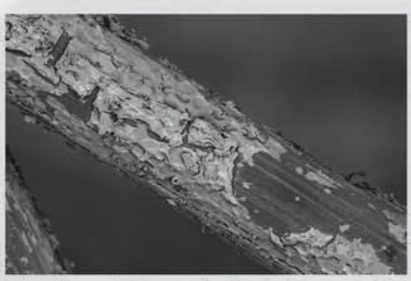


Channeling contrast in polychristalline silicon imaged with AsB® detector. Grain contrast, grain size, twins and grain boundaries are clearly visible in this imaging mode to determine the growth process.



The rim of a euro cent coin, imaged with the AsB® detector in 3D mode. The 3-dimensional surface modeling system within MERLIN® enables fast, accurate measurements which can be executed simultaneously during imaging.



In-lens SE image of a carbon nanotube. The optimized setup of the MERLIN® system regarding vibration reduction for high resolution imaging combined with highest ease of use gives even novice users the possibility to achieve expert results.

Carl Zeiss SMT
Nano Technology Systems Division

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

In copertina: Nanoparticelle di MgH2 decorate con nanocluster di Pd. Le nanoparticelle sono state sintetizzate per Inert Gas Condensation a partire da Mg e successivamente idrurate a 365°C e 3,5 bar di H2
di Annalisa Aurora

Indice

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:

a) mediante carta di credito dal sito www.sism.it
b) 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
c) mediante bonifico bancario intestato a S.I.S.M.
codice IBAN IT44V0100538880000000023074
Presso BNL-Anguillara S.
Causale: "NOME del SOCIO"

SEDE SOCIALE

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SOCIETÀ ITALIANA SCIENZE MICROSCOPICHE

Richiesta di associazione

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)

Titolo, Nome e Cognome

Data di nascita

Titolo di studio e qualifica

Tipo di istituzione

❑ Università ❑ CNR ❑ Industria ❑ Commerciale ❑ Altro ente pubblico di ricerca

Istituto/Ente/Ditta

Dipartimento

Indirizzo

Città CAP

Telefono Fax E-mail

Indirizzo cui inviare la corrispondenza, se diverso dal precedente

Settore di attività

❑ Biomedico ❑ Scienza dei materiali ❑ Commerciale ❑ Altro (specificare) _____

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.

❑ Chiedo che il mio indirizzo privato non compaia nell'annuario EMS
❑ Chiedo che il mio numero di telefono/fax non compaia nell'annuario EMS

Data _____ Firma _____

Inviare via fax a:
Dott.ssa Amelia Montone
ENEA, Dipartimento Tecnologie Fisiche e Nuovi Materiali C.R. Casaccia, Via Anguillarese, 301 00123 Roma
Tel +39.06.30484762/4764 Fax +39.06.30483176

Editoriale

Cari Amici,
quest'anno si terrà l'International Microscopy Congress a Rio de Janeiro dal 19 al 24 settembre 2010, vi ricordo che la scadenza per gli abstract è il 15 Aprile; inoltre, sono state bandite molte borse di studio per i giovani. Per maggiori informazioni potete consultare il sito: *www.imc17.com*. Durante questo Congresso si terrà l'Assemblea Generale dell'IFSM, alla quale parteciperà la nostra Società che ha diritto a due voti.
Rimanendo nell'ambito internazionale, vi ricordo che il prossimo MCM 2011, 10th Multinational Congress on Microscopy 2011, del quale avete recentemente ricevuto la splendida locandina, si terrà ad Urbino dal 4 al 9 Settembre 2011. È grande la nostra soddisfazione per l'andamento dell'organizzazione del Congresso, al quale hanno aderito con entusiasmo tutte le Società del Multinational; l'International Advisory Board è stato formato e sta già lavorando ai *topics* del Congresso. Il Congresso, organizzato da Elisabetta Falcieri, prevede un Workshop precongressuale ad Ancona sulla Tomografia e ricostruzione 3D, mentre ad Urbino il Congresso prevede sessioni sulla Strumentazione e Metodologia, sulla Scienza dei Materiali e sulla Biologia.
Quest'anno, oltre ad occuparsi dell'organizzazione scientifica del Congresso, la SISM organizzerà i seguenti eventi:
il Workshop "Contributi delle microscopie allo sviluppo delle nanotecnologie in campo biomedico: nanodrug delivery", che si terrà a Roma all'Istituto Superiore di Sanità il 12 Maggio 2010;
la "Scuola introduttiva teorico-pratica di Microscopia a Scansione di Sonda", che si svolgerà a Bologna, all'Istituto ISMN del CNR, dal 17 al 21 maggio 2010;
la "Scuola teorico-pratica di Microscopia Elettronica in Trasmissione nella Scienza dei Materiali", sempre a Bologna, all'Istituto IMM del CNR, a Novembre 2010.
Per gli aggiornamenti sulle attività SISM vi invito a consultare frequentemente il nostro sito web.
Vorrei ringraziare Roberto Balboni per aver gestito in modo ottimale il sito in questo passato biennio; tra breve la gestione passerà ad Andrea Tombesi, uno dei nuovi membri del Consiglio direttivo.
Per fare in modo che possiate ricevere tutte le informazioni SISM, chiedo a tutti i Soci di aggiornare i propri dati, specialmente l'indirizzo di posta elettronica, poiché molti indirizzi risultano non più validi.
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 non solo la cancellazione dall'elenco dei Soci SISM, ma anche dall'elenco dei Soci EMS.
Vi ricordo, infine, che la scadenza per partecipare al Premio SISM 2010 è il 30 Aprile 2010, aspettiamo con piacere le vostre richieste di partecipazione!
Ringrazio tutte le Ditte che ci hanno supportato lo scorso anno e che hanno già deciso di supportarci anche per il 2010.
Vi lascio alla lettura della Rivista che Manuela Malatesta sta gestendo con grande professionalità.

Amelia Montone

Editoriale

Cari Soci,
eccomi di nuovo a voi, in veste di direttore responsabile di Microscopie.
Due anni fa, quando iniziai questa avventura, ero allo stesso tempo timorosa ed eccitata, ma non ero certa che questo incarico mi avrebbe appassionato e, soprattutto, che mi sarebbe stata data l'opportunità di continuare per un secondo mandato. In questi due anni ho imparato molte cose, perché gestire la composizione di una rivista non significa semplicemente decidere la successione delle rubriche e degli articoli, ma implica relazionarsi con gli autori e gli inserzionisti, collaborare con i professionisti del-*l'editing* e della stampa, vedere e vivere un aspetto importante del nostro lavoro di ricercatori (la pubblicazione di articoli) da una prospettiva non usuale.
È stata un'esperienza che mi ha arricchito culturalmente, professionalmente ed umanamente, e spero che i prossimi due anni siano altrettanto proficui. Ringrazio di cuore tutti Voi ed i Colleghi del Consiglio Direttivo per l'apprezzamento per il lavoro già svolto e per la fiducia accordatami.
Come avrete notato, il numero degli articoli scientifici pubblicati su Microscopie sta progressivamente, seppur lentamente, crescendo e farò del mio meglio per mantenere e migliorare la qualità della nostra rivista. Ritengo, infatti, che anche attraverso la pubblicazione di buoni lavori originali si possa realizzare uno dei compiti centrali di una Società come la nostra: la formazione di una giusta coscienza scientifica nei giovani ricercatori.
La scrittura di un lavoro scientifico rappresenta il momento conclusivo e fondamentale dell'attività di un ricercatore; tuttavia, non è raro che i giovani, dopo aver svolto gran parte del lavoro sperimentale, partecipino solo marginalmente alla stesura del testo scientifico che, destinato alla dura competizione delle riviste internazionali, viene gestito da ricercatori più "anziani" ed esperti. Microscopie può rappresentare un buon banco di prova per i nostri giovani. Penso che voi, giovani Soci, dobbiate approfittare di questa occasione: sviluppate la vostra capacità di elaborare dati e discutere idee, assaporate il gusto della presentazione stilisticamente personale ed efficace del vostro lavoro sperimentale, coltivate la vostra onestà intellettuale senza la quale qualsiasi lavoro perde significato.
La nostra Società è certo in grado, e deve sentire il dovere, di contribuire a questa crescita, attraverso le sue iniziative didattico-formative e l'utilizzo di Microscopie anche come sede di proficuo confronto.
I nostri giovani Soci contribuiranno, anche quest'anno, ad arricchire la nostra rivista con le loro affascinanti immagini, proposte per il concorso "In copertina su Microscopie"; come già avvenuto nel 2009, una delle immagini sarà dedicata alle scienze dei materiali, l'altra alle scienze biomediche. Il successo della prima edizione del

concorso ha, infatti, indotto il Direttivo a riproporlo, come forma - forse non proprio "canonica" ma certo stimolante - di promozione delle scienze microscopiche.
Per finire, è doveroso ricordare che, nel 2011, il Multinational Congress on Microscopy si terrà ad Urbino, organizzato per la SISM da Elisabetta Falcieri. Si tratta di un grande evento che richiederà l'impegno non solo degli organizzatori, ma dell'intera Società che dovrà esprimere al meglio tutte le proprie capacità. In questo contesto, Microscopie svolgerà il suo ruolo, nell'informare i Soci sullo stato di avanzamento dei lavori di preparazione, sulle tematiche scientifiche del congresso e sulle opportunità logistiche offerte dalla splendida città marchigiana. L'attività promozionale da parte di Microscopie sarà un piccolo contributo agli sforzi degli organizzatori locali e potrà essere efficace solo se tutti i microscopisti Italiani sapranno rispondere con generosità ed orgoglio, inviando numerosi i loro più significativi contributi scientifici.

Manuela Malatesta

Consiglio direttivo della SISM

Verbale della riunione dell'11 dicembre 2009

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

Il giorno 11 dicembre 2009 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. Risultati elezioni per il rinnovo dei componenti il CD 2010-2011

3. Situazione economica della Società.

4. Attività SISM 2009 e discussione attività 2010

5. Organizzazione MCM2011

6. Sito web

7. Premio SISM 2010

8. Vincitori dei Premi Carla Milanesi

9. Approvazione ammissione nuovi Soci

10. Varie ed eventuali

Sono presenti: *Roberto Balboni*, *Elisabetta Falcieri*, *Guido Macchiarelli*, *Mario Raspanti e Amelia Montone.*
Assenti giustificati: *Alberto Diaspro e Manuela Malatesta.*
Presiede *Amelia Montone*; svolge le funzioni di segretario verbalizzante *Roberto Balboni*.

Alle ore 14 il CD sarà a disposizione dei Rappresentanti delle Ditte per illustrare le iniziative e le attività del 2010 ed unitamente al Comitato scientifico dell'MCM2011 presenterà lo stato organizzativo del 10th Multinational Congress on Microscopy (MCM 2011) che si terrà ad Urbino dal 4 al 9 Settembre 2011.
Prima dell'inizio della riunione si decide di comune accordo di spostare il Punto 5 in coda all'O.d.G, per permettere la partecipazione del prof. Paolo Mengucci e del dott. Giuseppe Arancia, membri del comitato organizzatore del MCM2011.

1. Il verbale della riunione del Direttivo del 29 giugno 2009 viene approvato all'unanimità.

2. Il Presidente dà lettura del verbale della commissione elettorale.
   L'esito della votazione per l'elezione del Presidente è il seguente:

MONTONE	voti	54
Schede bianche	voti	1

   Pertanto risulta eletta quale Presidente della Società Italiana di Scienze Microscopiche per il biennio 2010-2011: Amelia Montone.
   L'esito della votazione per l'elezione dei Consiglieri è il seguente:

BALBONI	voti	37
FALCIERI	voti	34
MALATESTA	voti	30
TOMBESI	voti	30
BISCARINI	voti	20
RASPANTI	voti	19
MACCHIARELLI	voti	14
MONTONE	voti	3
BOZZUTO	voti	1
FABBRI	voti	1
GRILLO	voti	1
MENGUCCI	voti	1
Schede bianche		1

Pertanto, in base all'art. 8 dello Statuto che recita "…..I Consiglieri rappresentano entrambi i settori della Società in numero non inferiore a due per settore." e dell'art. 12 che recita "…..Risultano eletti Consiglieri i primi sei Soci che abbiano avuto il maggior numero dei voti, salvo quanto disposto dall'art. 8.", risultano eletti quali Consiglieri della Società Italiana di Scienze Microscopiche per il biennio 2010-2011: *Roberto Balboni*, *Elisabetta Falcieri*, *Manuela Malatesta*, *Andrea Tombesi*, *Fabio Biscarini*, *Mario Raspanti*.

Il Presidente si rammarica per la mancata conferma alla carica di consigliere del prof. Guido Macchiarelli, lo ringrazia per l'importante contributo dato alla Società e auspica che la collaborazione impostata nei due anni di partecipazione al Consiglio possa proseguire anche attraverso il suo contributo dall'esterno. Guido Macchiarelli ringrazia il Presidente e assicura che la collaborazione con le iniziative SISM potrà continuare anche senza una sua presenza all'interno del Direttivo.

Il Presidente si congratula con i consiglieri riconfermati, e dà il benvenuto ai neoconsiglieri Tombesi e Biscarini, oggi non presenti a causa di precedenti impegni.

Macchiarelli formula una proposta di modifica dello statuto per il Direttivo entrante, allo scopo di dare continuità al direttivo eletto, in quanto ritiene due anni un periodo troppo limitato; propone di non limitare a due anni il mandato. Il Direttivo ritiene che una tale modifica debba essere ben ponderata e discussa all'interno della Società, anche sulla base delle precedenti esperienze.

3. Il Presidente illustra la situazione economica della Società. Le entrate provengono principalmente dalle scuole SISM, le uscite dalle quote associative all'EMS e IFSM, dalle spese per redazione, stampa e spedizione della rivista, e dai vari Premi SISM erogati. Il Consiglio approva la relazione all'unanimità.

   Macchiarelli formula la proposta di pubblicazione della rivista on-line, che potrebbe avere incidenza anche sui costi di redazione e stampa. Segue una breve discussione fra i consiglieri presenti: la proposta è ritenuta interessante, ma va ben progettata anche in vista di un rinnovo del sito web; viene comunque espresso il desiderio di mantenere un numero minimo di copie cartacee. Si decide pertanto di approfondire il tema in una successiva riunione del Direttivo.

4. Il Presidente relaziona sull'ottima riuscita delle attività del 2009.
   Per quanto riguarda le attività per l'anno 2010 la Società propone:
   - Workshop "Contributi delle microscopie allo sviluppo delle nanotecnologie in campo biomedico: nanodrug delivery",
     Roma, Istituto Superiore di Sanità, 12 Maggio 2010
     Organizzata dalla dott.ssa Agnese Molinari,
   - "Scuola introduttiva teorico-pratica di Microscopia a Scansione di Sonda" Bologna, CNR, Istituto ISMN, 17-21 maggio 2010
     Organizzata dal dott. Fabio Biscarini,
   - Scuola teorico-pratica di "Microscopia Elettronica in Trasmissione nella Scienza dei Materiali" Bologna, CNR, Istituto IMM, da 10 a 15 giorni, Novembre 2010.
     Organizzata da dott. Roberto Balboni e dott. Andrea Migliori.

6. Balboni illustra una breve proposta di modifica con l'obiettivo della costruzione di un sito facilmente

aggiornabile in futuro, e che richieda l'intervento del manutentore solo per aggiornamenti strutturali. Da un'analisi con l'attuale gestore si è deciso di sviluppare il nuovo sito su piattaforma PHP/MySQL. L'aspetto più oneroso è la gestione degli accessi e notifiche fra le quali anche i pagamenti, che non è attualmente prevista su tale piattaforma, così come la predisposizione di un sistema automatico per la gestione degli eventi (pagine delle scuole, iscrizioni e notifiche automatiche, …)
Il costo di massima per una modifica di questo tipo si aggira attorno a 2.500-3.500 € in funzione dei servizi. Sulla base di queste richieste verranno chiesti preventivi.

7. Il Presidente propone, come di consueto, di bandire il premio SISM per l'anno 2010, che consista di due borse: una per il settore biomedico e l'altra per il settore scienza dei materiali. Il Direttivo approva l'emissione del relativo bando, secondo le consuete modalità, ma decide di non vincolarlo alla partecipazione al Congresso di Rio, ritenendo la stessa un obbligo eccessivamente oneroso.

8. Viste le domande pervenute, visto il regolamento di partecipazione, il Direttivo, dopo aver valutato i contributi, presentati al MC 2009 (Microscopy Conference 2009) a Graz in Austria, dei partecipanti al Premio, decide all'unanimità di assegnare il Premio Carla Milanesi 2009 alla Dott. ssa Maria PALMERINI per il settore biomedico e alla dott.ssa Regina CIANCIO per il settore materiali.

9. Il Direttivo ratifica l'ammissione dei soci:
Ing. Salvatore GUASTELLA
Dott.ssa Agnese MOLINARI
Dott.ssa Francesca NUCERA
Dott.ssa Veronica VASCOTTO
Dott. Andrea DI GIULIO
Dott. Juri RIMAURO
Dott.ssa Vanda GRANATO
Dott. Matteo ZUCCARELLO
Dott. Jacopo TROISI
Ing. Federico MICCIULLA

10. Nulla da discutere.

5. Il Presidente del MCM2011, Elisabetta Falcieri, relaziona al Direttivo sullo stato dell'organizzazione del Congresso, e sull'incontro tenutosi a Roma il 26 Ottobre 2009 con il Comitato Organizzatore composto dalla Dott.ssa Montone, dal prof. Mengucci, dal dott. Arancia, dalla Dott.ssa Molinari e dalla Prof.ssa Falcieri. Mengucci informa che l'università di Ancona sostiene l'organizzazione di un workshop pre-congressuale mettendo a disposizione la sede ed eventuali alloggi per studenti; viene proposta una tematica relativa alla tomografia e alla ricostruzione 3D considerata la trasversalità di tali argomenti fra le varie discipline.
Viene inoltre discussa la brochure di presentazione del Congresso e vengono presentate le planimetrie degli spazi a disposizione per le Ditte.
Amelia Montone informa che sta contattando le altre Società del Multinational e l'EMS per le candidature al Comitato Scientifico.

Alle ore 13:30, null'altro essendovi da deliberare, il Presidente dichiara chiusa la seduta.

Amelia Montone
Roberto Balboni
Elisabetta Falcieri
Guido Macchiarelli
Mario Raspanti

Premio SISM 2010

La Società Italiana Scienze Microscopiche (SISM) bandisce un Premio riservato a **due giovani ricercatori**, di età non superiore a 35 anni (al momento della scadenza del bando), che utilizzino le tecniche microscopiche in campo biomedico o in scienza dei materiali.

Il Premio verrà assegnato sulla base dell'insindacabile giudizio del Consiglio Direttivo SISM che, nello stilare le graduatorie di merito, terrà conto dei seguenti elementi di giudizio:

- Curriculum vitae, completo di elenco delle pubblicazioni e di una breve descrizione delle principali tematiche di ricerca che evidenzi l'importanza dell'impiego delle tecniche microscopiche nel contesto delle indagini affrontate dal candidato
- Qualità e numero delle pubblicazioni scientifiche inerenti alle tematiche di microscopia
- Iscrizione alla SISM (a parità di giudizio, l'iscrizione costituirà titolo preferenziale)

I due premi, dell'ammontare di **€ 1000,00** ciascuno, uno per l'**area biologica** ed uno per l'**area di scienza dei materiali**, verranno assegnati ai vincitori, che dovranno preparare un articolo rappresentativo della loro attività di ricerca da pubblicare sulla rivista "Microscopie".

Ai primi 5 classificati della graduatoria del settore biologico e di quella del settore di scienza dei materiali verrà offerta l'iscrizione gratuita alla SISM per due anni, in riconoscimento della pertinenza e dell'eccellenza del curriculum scientifico.

Chi desidera partecipare dovrà indicare il settore (biologico o di scienza dei materiali) che ritiene più affine alla propria ricerca, inviare curriculum vitae completo e quanto ritenga utile ai fini della valutazione entro il 30 APRILE 2010.
Tutta la documentazione deve essere inviata esclusivamente per E-mail al Presidente SISM all'indirizzo:

amelia.montone@enea.it

Al ricevimento della documentazione verrà data E-mail di conferma dell'avvenuta ricezione.

Elenco delle attività promosse dalla SISM nel 2010

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. Giornata di Studio

Contributi delle microscopie allo sviluppo delle nanotecnologie in campo biomedico: nanodrug delivery

Roma, Istituto Superiore di Sanità, 12 Maggio 2010

La giornata di studio, organizzata dal Dipartimento di Tecnologie e salute dell'Istituto Superiore di Sanità in collaborazione con la SISM, affronterà alcuni aspetti correlati all'impiego delle microscopie ottiche ed elettroniche nel campo delle nanotecnologie con particolare riferimento al settore della nanomedicina (drug delivery). La giornata è rivolta sia a ricercatori, studenti e tecnici interessati alla microscopia sia a chi opera nel campo della nanomedicina. Essa prevede una prima parte in cui esperti nel settore terranno relazioni ad invito, ed una seconda parte in cui verranno presentate relazioni orali selezionate tra i contributi liberi dei vari partecipanti; si prevede inoltre una sessione poster.
Per questa iniziativa verrà richiesto al Ministero della Salute l'accreditamento nell'ambito del Programma Educazione Continua di Medicina (ECM).
Per informazioni: Dott.ssa Agnese Molinari (agnese.molinari@iss.it)

2. Scuola teorico-pratica

Scuola introduttiva teorico-pratica di Microscopia a Scansione di Sonda

Bologna, CNR-ISMN, ISOF, 17-21 Maggio 2010

La scuola introduttiva teorico-pratica di Microscopia a Scansione di Sonda (Scanning Probe Microscopy - SPM) si terrà presso l'Area della Ricerca del CNR di Bologna, Via Gobetti 101, dal 17 al 21 maggio 2010. La scuola è organizzata dai ricercatori CNR dell'Istituto per lo Studio dei Materiali Nanostrutturati (ISMN) e dell'Istituto per la Sintesi Organica e la Fotoreattività (ISOF) tutti attivi in SPM. L'obiettivo è introdurre gli aspetti teorici e sperimentali delle tecniche più diffuse della microscopia SPM. La scuola è indirizzata alle persone che non hanno conoscenza diretta o pratica della microscopia SPM e sono interessati a conoscerla o ad usarla. È richiesta una preparazione scientifica di base. Due giorni (Lunedì-17/05 e Martedì-18/05) saranno dedicati alla teoria senza restrizione nel numero di studenti (max 70). I restanti giorni (Mercoledì-19/05, Giovedì-20/05 e Venerdì-21/05) saranno dedicati ad attività sperimentale, con un massimo di 12 studenti a rotazione su sei microscopi diversi: AFM, STM, UHV-STM, AFM in liquido. E' previsto un modulo di preparazione campioni. E' possibile iscriversi alla sola parte teorica. Le lezioni e i laboratori saranno in Inglese in presenza di studenti e giovani ricercatori stranieri. La scadenza per le domande di partecipazione alla scuola è il 15/3/2010.
Docenti: Dott. Fabio Biscarini (f.biscarini@bo.ismn.cnr.it), direttore della Scuola; Cristiano Albonetti (c.albonetti@bo.ismn.cnr.it), Eva Bystrenova, Massimiliano Cavallini, Alessandro Gambardella, Andrea Liscio, Vincenzo Palermo, Francesco Valle.

3. Scuola teorico-pratica

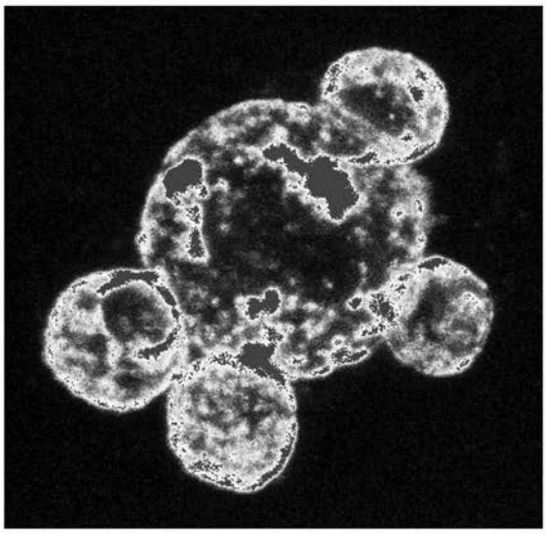
Scuola teorico-pratica di Microscopia Elettronica in Trasmissione in Scienza dei Materiali
Istituto CNR-IMM Bologna - Novembre 2010

La scuola, organizzata congiuntamente dalla SISM e dal CNR-IMM di Bologna, si rivolge a ricercatori e microscopisti che desiderano acquisire una qualificata introduzione alle tecniche di microsco-

pia elettronica in trasmissione applicata alla Scienza dei Materiali. Ai partecipanti verrà fornito un quadro teorico di base della disciplina e una descrizione delle principali applicazioni nell'indagine strutturale ed analitica. Gli argomenti trattati saranno: ottica e diffrazione elettronica, elementi di cristallografia, teoria del contrasto, risoluzione atomica con tecniche di imaging coerenti (HREM) e incoerenti (STEM con rivelatore HAADF), olografia elettronica, tecnica CBED, metodi analitici (EDX e EELS).
La scuola sarà strutturata in una parte teorica, durante la prima settimana, ed una parte pratica la seconda settimana. Sarà possibile la partecipazione all'intero corso, o alla sola parte teorica.
Per informazioni: Dott. Andrea Migliori (migliori@bo.imm.cnr.it), Dott. Roberto Balboni (balboni@bo.imm.cnr.it)

Corsi SISM

S.I.S.M.



CONTRIBUTI DELLE MICROSCOPIE ALLO SVILUPPO DELLE NANOTECNOLOGIE IN CAMPO BIOMEDICO: NANODRUG DELIVERY

Roma, 12 maggio 2010

Aula Pocchiari
Istituto Superiore di Sanità
Viale Regina Elena 299, 00161 Roma

Direzione scientifica:
Velio Macellari, Agnese Molinari
(ISS , Roma)

Segreteria organizzativa:
Franca Grisanti, Monica Brocco
Cristina Quattrini
(ISS , Roma)

Segreteria scientifica:
Giuseppe Arancia, Amelia Montone,
Stefania Meschini, Annarita Stringaro
(ISS , Enea Casaccia - Roma)

Supporto tecnico :
Giuseppina Bozzuto, Marisa Colone,
Maria Condello, Giuseppe Formisano,
Laura Toccacieli
(ISS , Roma)

Con il supporto di:

FEI COMPANY — ***FEI Company Srl***

Leica — ***Leica Microsystems***

JEOL — ***Jeol Italia Spa***

assing — ***Assing Spa***

La giornata di studio, organizzata dal Dipartimento Tecnologie e salute dell'Istituto Superiore di Sanità in collaborazione con la Società Italiana di Scienze Microscopiche (SISM), affronterà alcuni aspetti correlati all'impiego delle microscopie ottiche ed elettroniche nel campo delle nanotecnologie con particolare riferimento al settore della nanomedicina (drug delivery).

La giornata è rivolta sia a ricercatori, studenti e tecnici interessati alla microscopia sia a chi opera nel campo della nanomedicina. Essa prevede una prima parte in cui esperti nel settore terranno relazioni ad invito ed una seconda parte in cui verranno presentati relazioni orali selezionate tra i contributi liberi dei vari partecipanti; si prevede inoltre una sessione poster.

Pur prediligendo contributi che associano l'impiego delle microscopie allo studio del nanodrugdelivery, sono graditi anche lavori in cui vengono utilizzati altri metodi di indagine.

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

Il Corso è stato accreditato presso il Ministero della Salute nell'ambito del Programma "Educazione Continua in Medicina" (ECM).

Programma

9:00 - Registrazione e saluto ai partecipanti

- La microscopia elettronica nella caratterizzazione dei nanomateriali per uso biomedico
Andrea Falqui – Istituto Italiano di Tecnologia, Genova.

- Microscopia a raggi X: principi di base e sue potenziali applicazioni nel campo del drug delivery
Stefano Lagomarsino - Istituto Fotonica e Nanotecnologie, CNR, Roma.

- Coated nanogold and the brain – A Detailed Microscopic Distribution Study
Silke Krol - CBM – Cluster in Biomedicine, AREA Science Park, Trieste.

Pausa caffè

- Caratterizzazione chimico fisica e biologica di liposomi cationici
Giovanna Mancini - CNR, Istituto di Metodologie Chimiche e Dipartimento di Chimica - Università di Roma "Sapienza".

- **Effetto delle proprietà chimico-fisiche sull'internalizzazione di nanovettori in cellule endoteliali**
Paolo Antonio Netti - Centro di Ricerca Interdipartimentale sui Biomateriali (CRIB) e *Istituto Italiano di Tecnologia, Genova.*

Interventi Ditte

Pausa pranzo

!4:00 - Sessione poster

15:00 - Relazioni orali selezionate

18:00 - Chiusura dei lavori

Iscrizione

L'abstract e la scheda di iscrizione devono essere inviate entro il **30 Aprile 2010** per e-mail (nanodrug2010ws@iss.it), unitamente alla copia del versamento della quota di iscrizione.

E' inoltre possibile effettuare l'iscrizione online al sito www.sism.it .

Le **quote di iscrizione** comprendono l'accesso ai lavori, il materiale informativo, la pausa caffè ed il pranzo.

SOCIO SISM[1]: € 50 + IVA 20%

NON SOCIO SISM: € 80 + IVA 20%

[1] l'offerta è da considerarsi valida per i soci che risultano iscritti al **31 Dicembre 2009**

A fronte del pagamento sarà rilasciata regolare fattura. Si ricorda che per i dipendenti di Enti Pubblici la quota è esente da IVA (art. 10 DPR 633/72).

Le quote d'iscrizione possono essere versate attraverso:

1) **Carta di credito** (dal sito www.sism.it)

2) **Bonifico bancario** intestato a

S.I.S.M.

IBAN: IT 44 V 01005 38880 0000 00023074 presso BNL-Anguillara Sabazia (ROMA)

Causale: "**Cognome del partecipante + RM1"**

3) **Assegno bancario non trasferibile**

intestato a **S.I.S.M.**, da inviare a:

Dott.ssa **Amelia Montone**,

ENEA, Dipartimento Tecnologie Fisiche e Nuovi Materiali, C.R. Casaccia, Via Anguillarese, 301, 00123 Roma

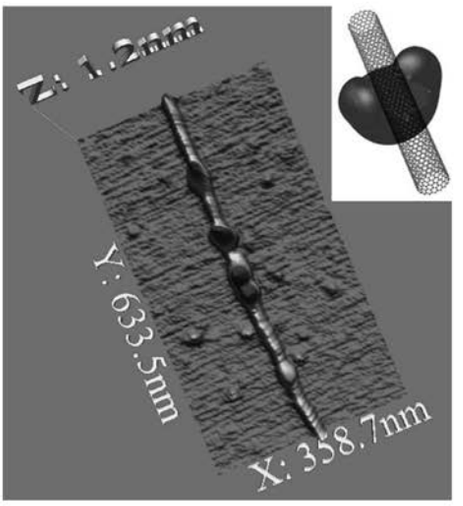
Chi farà richiesta di associazione alla SISM sarà esonerato dal versamento della quota associativa per l'anno 2010

Dimensioni poster: larghezza 60 cm, altezza 90 cm.

Istruzioni per l'abstract : vedi di seguito

Info: nanodrug2010ws@iss.it

Corsi SISM



Scuola introduttiva teorico- pratica di Microscopia a Scansione di Sonda

Bologna, 17 – 21 Maggio 2010

Consiglio Nazionale delle Ricerche

Area della Ricerca di Bologna
Via Gobetti 101 – 40129 Bologna

Direzione scientifica
Fabio Biscarini
(CNR-ISMN Bologna)

Comitato organizzatore
Cristiano Albonetti, Eva Bystrenova, Massimiliano Cavallini, Alessandro Gambardella, Andrea Liscio, Vincenzo Palermo, Francesco Valle

Con il patrocinio di

Dipartimento Progettazione Molecolare

Con il supporto di

Pra.Ma. NT-MDT FEI Tassing JEOL Veeco Gambetti 2M STRUMENTI BRUKER

Informazioni Generali

La società **SISM** in collaborazione con l'Istituto per lo Studio dei Materiali Nanostrutturati (**ISMN**) e l'Istituto per la Sintesi Organica e la Fotoreattività (**ISOF**) del **CNR** (Area della Ricerca di Bologna) organizza una scuola introduttiva teorico-pratica di Microscopia a Scansione di Sonda (Scanning Probe Microscopy - **SPM**). L'obiettivo didattico è quello di introdurre i partecipanti agli aspetti teorici e sperimentali delle tecniche più diffuse della microscopia SPM: la Microscopia ad effetto Tunnel in Scansione (**STM**) e la Microscopia a Forza Atomica (**AFM**). La scuola si rivolge a coloro che non hanno conoscenza diretta o pratica della microscopia SPM, ma sono interessati a conoscerla o ad usarla. E' richiesta una preparazione scientifica di base. I primi due giorni (**Lunedì-17/05** e **Martedì-18/05**) saranno dedicati alla teoria delle microscopie SPM e ai metodi di analisi delle immagini SPM (Modulo Teorico). I restanti giorni (**Mercoledì-19/05**, **Giovedì-20/05** e **Venerdì-21/05**) saranno dedicati alle attività sperimentali (Modulo Sperimentale), con rotazione dei partecipanti su sei microscopi diversi: AFM, STM, UHV-STM, AFM in liquido. E' previsto un seminario dedicato alla preparazione campioni. E' possibile iscriversi al solo modulo teorico. Le lezioni e i laboratori saranno in Inglese in presenza di studenti e giovani ricercatori stranieri. La scadenza per le domande di partecipazione alla scuola è fissata al **15/3/2010**. La parte sperimentale prevede l'osservazione di campioni organici, inorganici, biologici e nanostrutturati. Durante i giorni dedicati al modulo teorico, le principali ditte leader nel settore della microscopia a sonda in scansione presenteranno le ultime novità strumentali del settore.
Alla conclusione della scuola, i partecipanti avranno gli strumenti teorico-sperimentali per comprendere ed utilizzare le microscopie STM e AFM.

Il modulo teorico prevede un numero massimo di **70 partecipanti**
Il modulo sperimentale prevede un numero massimo di **12 partecipanti**[1].
È richiesto un numero **minimo** di **8 partecipanti** per attivare il corso.

Per ulteriori informazioni rivolgersi a:
Prof. Fabio Biscarini f.biscarini@bo.ismn.cnr.it
Tel: 0516398522
Dr. Cristiano Albonetti c.albonetti@bo.ismn.cnr.it
Tel: 0516398523

[1]In base al numero di iscritti e alla disponibilità dei microscopi esposti dagli sponsors, il numero massimo dei partecipanti potrà essere esteso. La scelta sarà a discrezione dei responsabili della scuola bospm'10.

Programma

Lunedì
9:30 **Registrazione e saluto ai partecipanti** – Prof. F. Biscarini (ISMN)
9:45 – 11:15 **Introduzione alle microscopie a sonda in scansione (SPM): elementi comuni e costruttivi** – Dr. C. Albonetti (ISMN)
11:15 – 11:30 **Coffee break**
11:30 – 13:00 **La microscopia in scansione ad effetto tunnel (STM)** – Dr. A. Gambardella (ISMN)
13:00 – 14:00 **Pranzo Mensa CNR**
14:00 – 15:45 **La microscopia a forza atomica: contatto, non-contatto e contatto intermittente** – Dr. V. Palermo e Dr. A. Liscio (ISOF)
15:45 – 16:00 **Coffee break**
16:00 – 17:45 **Metodologie di analisi delle immagini SPM (I)** – Prof. F. Biscarini e Dr. C. Albonetti (ISMN)
17:00-18:00: **Innovazione strumentale** - Sponsors

Martedì
9:30 – 11:15 **La microscopia a forza atomica in ambiente liquido applicata a campioni biologici** – Dr. F. Valle (ISMN)
11:15 – 11:30 **Coffee break**
11:30 – 13:00 **Metodologie di analisi delle immagini SPM (II)** – Prof. F. Biscarini e Dr. C. Albonetti (ISMN)
13:00 – 14:00 **Pranzo Mensa CNR**
14:00 – 15:45 **Preparazione campioni per la microscopia a sonda** – Dr. E. Bystrenova e Dr. Massimiliano Cavallini (ISMN)
15:45 – 16:00 **Coffee break**
16:00 – 17:00 **Laboratorio interattivo analisi immagini** – Prof. F. Biscarini e Dr. C. Albonetti (ISMN)
17:00-18:00: **Innovazione strumentale** - Sponsors

Mercoledì – Venerdì
9:30 - 18:00 **Esercitazioni pratiche su Microscopio ad effetto Tunnel in Scansione (STM) e Microscopio a Forza Atomica (AFM)**[1,2]
11:15 – 11:30 **Coffee break**
13:00 – 14:00 **Pranzo Mensa CNR**
15:45 – 16:00 **Coffee break**

[1]**Lavoro a coppie e a rotazione**
[2]**Possibile utilizzo dei microscopi in esposizione**

Iscrizione

La scheda di iscrizione deve essere inviata entro il **15 Marzo 2010** per e-mail (f.biscarini@bo.ismn.cnr.it, c.albonetti@bo.ismn.cnr.it) o per fax (**051-6398539**), unitamente alla copia del versamento della quota di iscrizione.

Le **quote di iscrizione** comprendono l'accesso ai lavori, il materiale didattico, i coffee breaks, i pranzi alla mensa CNR e la cena sociale (**solo parte sperimentale**).

Modulo Teorico: 2 giorni (17-18 Maggio 2010)

SOCIO SISM[1]: € 200 + IVA 20%

NON SOCIO SISM: € 300 + IVA 20%

Modulo Teorico + Modulo Sperimentale: 5 giorni (17-21 Maggio 2010)

SOCIO SISM[1]: € 600 + IVA 20%

NON SOCIO SISM: € 700 + IVA 20%

Per i *non strutturati* (assegnisti di ricerca, dottorandi e contrattisti a tempo determinato) **riduzione del 20%** sulle quote di iscrizione calcolate al netto dell'IVA.

[1] L'offerta è da considerarsi valida per i soci che risultano iscritti al **31 Dicembre 2009**

A fronte del pagamento sarà rilasciata regolare fattura. Si ricorda che per i dipendenti di Enti Pubblici la quota è esente da IVA (art. 10 DPR 633/72)

Le quote d'iscrizione possono essere versate attraverso:

1) **Carta di credito** (dal sito www.sism.it)

2) **Bonifico bancario** intestato a

S.I.S.M.

IBAN: IT 44 V 01005 38880 0000 00023074
presso BNL-Anguillara Sabazia (ROMA)
Causale: "**Cognome del partecipante + BOSPM"**

3) **Assegno bancario non trasferibile**

intestato a **S.I.S.M.**, da inviare a:
Dott.ssa **Amelia Montone**,

ENEA, Dipartimento Tecnologie Fisiche e Nuovi Materiali, C.R. Casaccia, Via Anguillarese, 301, 00123 Roma

Chi farà richiesta di associazione alla SISM sarà esonerato dal versamento della quota associativa per l'anno 2010.

Resoconto della scuola SISM

Scuola teorico-pratica di Microscopia Elettronica a Scansione applicata ai materiali nanostrutturati

12-16 ottobre 2009, Roma, ENEA, Centro Ricerche Casaccia

Direzione scientifica: Amelia Montone (CR ENEA Casaccia)

La scuola, organizzata dalla SISM in collaborazione con l'ENEA, ha affrontato i principi della Microscopia Elettronica a Scansione (SEM) e le sue applicazioni nel campo della Scienza dei Materiali, con particolare riguardo ai materiali nanostrutturati. Più di 20 partecipanti hanno assistito alle lezioni teoriche e pratiche. Le relazioni e le esercitazioni pratiche sono state particolarmente apprezzate dai partecipanti per l'alto livello scientifico accompagnato dalla capacità di rendere chiari concetti complicati, vale la pena citarli tutti:
SEM: Struttura e funzionamento (M.Tonelli, CIGS Modena)
Elementi di ottica elettronica, interazione elettrone-materia (D. Mirabile Gattia, CR ENEA Casaccia, Roma)
Rivelatori e segnali nel SEM (M. Tonelli, CIGS Modena)
Il microscopio a scansione a ioni di elio: immagini di superficie con risoluzione subnanometrica (G. Lamedica, Assing)
The XHR SEM: Magellan -Extreme High Resolution (F. Tatti, FEI Italia)
Introduzione al Focused Ion Beam (G.C. Gazzadi, CNR-INFM S3, Modena)
La microanalisi a Raggi X (A. Aurora, CR ENEA Casaccia, Roma)
La preparazione dei campioni per l'osservazione al SEM (L. Pilloni, CR ENEA Casaccia, Roma)
Requisiti strumentali e operativi per ottimizzare la risoluzione nel SEM (M. Vittori Antisari, CR ENEA Casaccia, Roma)
SEM e tecniche analitiche correlate per l'analisi dei materiali nanostrutturati (M. Rossi, Università La Sapienza, Roma)
Nanotecnologie per la Conservazione del patrimonio artistico (M.Favaro, ICIS-CNR, Padova)
Applicazioni alle nanotecnologie nell'industria (P. Schiavuta, Associazione CIVEN, Venezia)
Deposizione indotta da fascio elettronico di nanofili (G.C. Gazzadi, CNR-INFM S3, Modena)
Esercitazioni pratiche (F. Pierdominici, A. Montone, L. Pilloni, CR ENEA Casaccia, Roma)

Si ringrazia l'Assing Spa, la Bruker AXS Srl, la FEI Italia e la Jeol Italia per aver supportato questa iniziativa e per aver partecipato attivamente al Corso portando strumentazione, materiale illustrativo e presentando le ultime novità sul SEM.
Si ringrazia, per l'ottima organizzazione del Corso il Comitato organizzatore: Annalisa Aurora, Patrizia Francesconi e Renzo Marazzi (CR ENEA Casaccia).

Amelia Montone

Resoconto del corso SISM

Tecnica ed applicazioni dei calchi vascolari (vascular corrosion cast)

3-4 dicembre 2009, Varese, Università dell'Insubria

Responsabili del corso: Mario Raspanti, Simone Sangiorgi

Il Corso SISM sulla "tecnica ed applicazione dei calchi vascolari" è stato organizzato congiuntamente dal Dipartimento di Morfologia Umana e dal Dipartimento di Scienze Chirurgiche dell'Università dell'Insubria a Varese facendo seguito alla "Corrosion Casting Conference" del 2008. Il corso ha affrontato i vari aspetti tecnici e metodologici dei calchi vascolari, una tecnica di imaging del microcircolo difficile e specialistica, praticata da pochi ricercatori a livello europeo, ma in grado di dare informazioni insostituibli sulla anatomia e la fisiologia dell'apparato circolatorio.
Nonostante l'improvvisa *défaillance* di due relatori, ha comunque potuto avvalersi dell'intervento di Simone Sangiorgi (Varese) sugli aspetti pratici, vantaggi, svantaggi ed artefatti dei calchi in metacrilato, di Eric Mayer (Zurigo) sui calchi in poliuretano e di Mario Raspanti (Varese) sulla visualizzazione, la ricostruzione 3D dei calchi ed i relativi problemi metrologici. Una seconda sessione applicativa ha visto interventi di Elias Polykiandrotis (Erlangen) sulla vascolarizzazione degli organi artificiali, di Hanna Jackowiak (Poznan) sull'architettura vascolare della cistifellea, di Guido Macchiarelli (L'Aquila) sul microcircolo dell'ovaio e di Alessandro Manelli (Imperia) sulla architettura vascolare del dito umano.
Il secondo giorno è stato interamente dedicato all'osservazione pratica dei preparati.
Si ringrazia per la disponibilità e la collaborazione tutto il Laboratorio di Morfologia Umana, il Sig. Franco Gialdinelli ed il Centro Grandi Strumenti dell'Università dell'Insubria che ha reso disponibile il SEM FEI XL 30-FEG usato per le osservazioni.

Mario Raspanti

Microscopy Conference 2009 – Graz

Al Congresso di Graz (30 Agosto - 4 Settembre 2009) quattro nostri giovani ricercatori sono stati premiati per i migliori poster, a riprova della qualità scientifica delle nuove leve della microscopia italiana.

Microscopy Conference 2009 in Graz MC 2009 Graz

30 August – 4 September 2009
Graz, Austria

Best Poster Award

On behalf of the International Programme Committee I am pleased to certify that

Laura Felisari

received a Best Poster Award of 250 €
for an outstanding scientific contribution
in the field of the Conference Topic
"SEM"

H. P. Karnthaler

Prof. H. Peter Karnthaler
President of MC 2009 Graz


Microscopy Conference 2009 in Graz
MC 2009 Graz
30 August – 4 September 2009
Graz, Austria
Best Poster Award
On behalf of the International Programme Committee I am pleased to certify that
Luca Ortolani
received a Best Poster Award of 250 €
for an outstanding scientific contribution
in the field of the Conference Topic
“Carbon based materials, soft materials, polymers”
Prof. H. Peter Karnthaler
President of MC 2009 Graz
ASEM

Microscopy Conference 2009 in Graz MC 2009 Graz

30 August – 4 September 2009
Graz, Austria

Best Poster Award

On behalf of the International Programme Committee I am pleased to certify that

Alessandra Zanola

received a Best Poster Award of 250 €
for an outstanding scientific contribution
in the field of the Conference Topic
"High-resolution light microscopy,
correlative light and electron microscopy"

H. P. Karnthaler

Prof. H. Peter Karnthaler
President of MC 2009 Graz

Vincitori del concorso "In copertina su Microscopie"

Anche per la seconda edizione del premio "In copertina su Microscopie", bandito lo scorso autunno ed indirizzato ai giovani Soci non strutturati della SISM, sono giunte in redazione diverse immagini suggestive, da parte di ricercatori impegnati sia nelle scienze dei materiali sia in biomedicina.

Le immagini sono state esaminate dai membri del Consiglio Direttivo che hanno espresso il loro giudizio e, tenendo conto soprattutto della qualità estetica, sono risultati vincitori la dottoressa **Annalisa Aurora** (ENEA C.R. Casaccia, Roma) per le Scienze dei Materiali e il dottor **Alberto Foglia** (Lugano-Paradiso, CH) per le Scienze Biomediche.

Sulla copertina di questo numero compare l'immagine di Annalisa Aurora, mentre quella di Alberto Foglia sarà pubblicata sul fascicolo di settembre: ai vincitori, che sono esonerati dal pagamento della quota sociale per il 2010, saranno inviate dieci copie del fascicolo sul quale compare la loro immagine premiata.

Eventi nazionali

Corso Teorico Pratico - Nanoparticelle: caratterizzazione e interazioni biologiche
24-26 Marzo 2010
Università degli Studi Milano Bicocca - Centro di Ricerca POLARIS
Per informazioni: polaris@unimib.it

Corso Teorico-Pratico di Ultramicrotomia e Crio-ultramicrotomia
17-21 maggio 2010
Università degli Studi di Milano - Fondazione Filarete
Per informazioni: barbara.martini@fondazionefilarete.com

64mo Congresso Nazionale della Società Italiana di Anatomia e Istologia
15-18 settembre 2010
Messina – Taormina
http://www.siaitaormina2010.com/

Eventi internazionali

2010

The NIBSC CEMOVIS course
April 12-16, 2010
National Institute for Biological Standards and Control (NIBSC), Hertfordshire, United Kingdom

Weinstein Cardiovascular Development Conference
EMS sponsored event (find more information on EMS sponsored events)
May 20-22, 2010
Royal Tropical Institute (Tropenmuseum), Amsterdam, The Netherlands
Organization: Heart Failure Research Center

E-MRS 2010 Spring Meeting: Quantitative Electron Microscopy for Research and Industry
EMS sponsored event (find more information on EMS sponsored events)
June 7-11, 2010
Congress Center, Strasbourg, France
Organization: European Materials Research Society (E-MRS)

SCANDEM 2010
June 8-10
Kista Forum, Stockholm, Sweden
Main organizer: Prof. Oleg Shupliakov, KI
Oleg.Shupliakov@ki.se

Past, present and future of (S)TEM and its applications
EMS sponsored event (find more information on EMS sponsored events)
A tribute to the work of Christian Colliex
June 9-11, 2010
CNRS headquarters, Paris, France
Organization: Laboratoire de Physique des Solides, Groupe STEM

MICROSCIENCE 2010
June 28 - July 1, 2010
ExCeL, London, UK
Organization: RMS

Workshop on Aberration Corrected STEM
July 3-5, 2010
SuperSTEM, Daresbury, United Kingdom

CPO-8
8th International Conference in Charged Particle Optics
July 12-16, 2010
Suntec Singapore Convention & Exhibition Centre, Singapore

Inter/Micro 2010 Microscopy Symposium
July 12-16, 2010
McCrone Research Institute, Chicago, USA

International LEEM PEEM Workshop (LEEMPEEM7)
August 8-13, 2010
Grand Hyatt Hotel in Manhattan, New York City, NY, USA

52nd Symposium of the Society for Histochemistry
Advanced imaging techniques in biomedicine: from molecules to organisms
September 1-4, 2010
Prague, Czech Republic
Organization: Society for Histochemistry

FEBS Advanced Course 2010
Microspectroscopy: Probing Protein Dynamics and Interactions in Living Cells
September 14-23, 2010
MicroSpectroscopy Centre (MSC) of the Wageningen University and the Microscopic Imaging Centre (MIC) of the Radboud University Nijmegen Medical Centre, The Netherlands

17th International Microscopy Congress (IMC17)
September 19-24, 2010
Rio de Janeiro, Brazil
Organization: SBMM, IFSM

2011

Microscopy Conference MC 2011
August 28 - September 2, 2011
Kiel, Germany
Organization: DGE, SCANDEM, PTMi

10th Multinational Congress on Microscopy (MCM 2011)
September 4-9, 2011
Urbino, Italy
Organization: ASEM, CSEM, CSMS, HSM, SISM, SSM, SDM

2012

15th European Microscopy Congress, EMC 2012
London, ExCel venue
Organization: EMS, RMS, EMAG at the ExCel site

Eventi internazionali

Bringing together, through all forms of Microscopy, the frontiers of Nanotechnologies and applications, Medicine and Life Sciences, Energy conversion, Environmental protection and much more...

The International Microscopy Congress (IMC) held every four years under the auspices of International Federation of Societies for Microscopy (IFSM) has been providing an international forum for the state of the art of microscopy at the frontiers of research and applications in Materials Sciences and in Life Sciences. The Brazilian Society for Microscopy and Microanalysis (SBMM) is proud to host the IMC17 in Rio de Janeiro, September 19-24, 2010.

The IFSM and SBMM invite you to submit an abstract for presentation at the IMC17 through the website

www.imc-17.com

Materials Science and Nanotechnology

- **M-1** Nanowires, Nanotubes and Particles
- **M-2** Graphene, fullerenes and carbon nanotubes
- **M-3** Surfaces, Interfaces, Thin Films and Coatings
- **M-4** Grain Boundaries and Defected Structures
- **M-5** Semiconductors and LSI device materials
- **M-6** Magnetic materials and super-conducting materials
- **M-7** Catalytic materials
- **M-8** Metals, alloys and phase transformations
- **M-9** Ceramics and inorganic materials including oxides
- **M10** Amorphous, disordered materials and quasicrystals
- **M-11** Polymers, molecular crystals and radiation sensitive materials
- **M-12** Materials related to nano-manufacturing technology
- **M-13** Composite and self-assembled materials
- **M-14** Porous materials
- **M-15** Geological and mineralogical materials
- **M-16** Microscopy of Biological, Biomimetic and Medical Materials
- **M-17** Industrial applications of microscopy
- **M-18** Forensic applications
- **M-19** In situ applications of microscopy
- **M-20** Materials in archeology and cultural heritage
- **M-21** Energy-related materials
- **M-22** Environmental-related materials and air-born particles

Life Sciences

- **L-1** Confocal and multifoton microscopy
- **L-2** High resolution light microscopy
- **L-3** Live cell imaging
- **L-4** Correlative light-electron microscopy
- **L-5** Advances in scanning electron microscopy
- **L-6** Scanning probe microscospy
- **L-7** Immuno-Electron microscopy
- **L-8** Microscopy cytochemistry
- **L-9** Analytical microscopy and biological nanoanalysis
- **L-10** Electron microscopy of cellular transport and dynamics
- **L-11** Three-dimensional reconstruction in electron microscopy
- **L-12** Cryoelectron microscopy
- **L-13** Plant science
- **L-14** Mycology and parasitology
- **L-15** Bacteriology and virology
- **L-16** Cell and tissue structure
- **L-17** Pathology
- **L-18** Hard tissues
- **L-19** Supramolecular structure
- **L-20** Structure of macromolecular complexes
- **L-21** Advances in biological sample preparation

Instrumentation and Techniques

- **I-1** Development in electron optical elements (guns, lens monochromator, filter, detector)
- **I-2** Advancing HR-TEM and HR-STEM
- **I-3** Advances in SEM and SIM
- **I-4** Quantitative electron diffraction techniques (CBED, EBSD)
- **I-5** Electron energy loss spectroscopy and energy-filtered imaging/mapping
- **I-6** Advances in X-ray/CL spectrometry and mapping
- **I-7** Electron tomography
- **I-8** Electron holography
- **I-9** In-situ and UHV electron microscopy
- **I-10** Environmental microscopy (SEM, TEM, SPM)
- **I-11** Surface microscopy (LEEM, PEEM, AEM, EPMA)
- **I-12** Scanning probe microscopy (STM, SFM, MFM)
- **I-13** Advanced optical microscopy (NSOM, Confocal microscopy)
- **I-14** Atom Probe Tomography
- **I-15** X-ray microscopy
- **I-16** Digital imaging and processing techniques
- **I-17** Remote electron microscopy
- **I-18** Advances in sample preparation techniques
- **I-19** Focused ion beam theory, application and techniques for physical and biological sciences
- **I-20** Advances in instrumentation and techniques
- **I-21** Lensless Imaging
- **I-22** Forty Years after the First EM Images of Single Atoms
- **I-23** Phase Contrast Devices theoretical concepts and applications

Eventi internazionali

Scholarships for IMC17

Dear EMS member,

During the recent EMS Board meeting it was decided to select MICROSCIENCE 2010 and SCANDEM 2010 as EMS Extensions for this year.

To support young researchers **15 scholarships** of each **250 Euro** will be awarded for participation at one of these meetings. Application deadline is the same deadline as the abstract submission deadline. Each applicant mustsubmit at least one abstract and be registered at the meeting. Copy ofabstract(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.

Also, **several scholarships** of each **500 Euro** will be awarded for participation at IMC17 in Rio de Janeiro. Application deadline is the same deadline as the original abstract submission deadline. 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
http://www.eurmicsoc.org/

Dear EMS members,

Following our earlier general mail on EMS scholarships for MICROSCIENCE 2010, SCANDEM 2010 and IMC17, it is our great pleasure that JEOL has offered to sponsor **10 extra EMS scholarships** for IMC17 in Rio de Janeiro. Same conditions and procedure apply (see also http://www.eurmicsoc.org/scholarships.htm).

Many thanks to JEOL and we're looking forward to your applications by April 15.

All the best,

Nick Schryvers
EMS Secretary
http://www.eurmicsoc.org/

Eventi internazionali

IFSM
International Federation of Societies for Microscopy
http://www.ifsm.uconn.edu/

Orsay, February 05, 2010

Christian COLLIEX
President

to all Presidents, National and Regional Societies for Microscopies

Dear colleagues and friends,

In these early days of year 2010, please receive first my best wishes for a healthful and successful new year for yourselves, personally and professionally, and for your societies.

As a matter of fact, as it regularly comes back every fourth year, 2010 will be a very important year for all of us, as it will provide us with the chance of joining again in a great environment for sharing new science and techniques, for developing new projects, for exchanging ideas, for friendly and warmly socializing and for gathering collections of nice memories.

From September 19 to September 24, our Brazilian colleagues under the chairmanship of Guillermo Solorzano and with the support of the Brazilian Society for Microscopy and Microanalysis (SBMM) and its president Karla Balzuweit, will host us in Rio de Janeiro, this exuberant and cosmopolitan city of the Latin America continent.

The 17th International Microscopy Conference will thus make its first visit to this continent after having touted all over the world during the past twenty years (Paris 1994, Cancun 1998, Durban 2002, Sapporo 2006). This choice has been made by your representatives during our last general assembly which took place in Sapporo, on the 6th of September 2006.

The Conference will be held at the Windsor Barra Convention Center and Hotel, located in the south part of Rio, in a quiet area, in front of the privileged Barra da Tijuca beach. I invite you to visit from now the conference website at http://www.imc17.com/ where you will find a first set of quite useful information about Rio and the conference site.

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The scientific programme is actively under construction. The plenary speakers of the conference have been chosen and invited. On my own side, as the IFSM President, I have brought together a panel of three very distinguished and world leading experts in different aspects of microscopy and applications for the Presidential Symposium which will conclude IMC17 on Friday September 24, 2010 (see the website). Another substantial implication of IFSM is the delivery of grants for selected students, all of them will therefore be invited to attend a special pre-conference School which is devoted to them on Sunday September 19, at the beginning of the meeting. IFSM will offer 50 grants for international students, SBMM will also offer 50 grants for Brazilian students, 25 for Latin American students and 10 to African ones. More detailed information about application for grants, selection of candidates and pre-conference School program will soon be available on the website. Finally the 2010 Cowley Prize and Medal, created in 2006 with the Cowley Medal Fund and then awarded to Sumio Iijima, will be presented during IMC17 conference.

Now, I wish to focus on the IFSM general assembly which will be held during the Conference. In the continuation of the last one in Sapporo (the minutes of which will be soon mailed to you by the General Secretary), this assembly, beyond providing news about IFSM life and activities, will have three major tasks:

(i) revise its constitution in order to combine by-laws and statutes. From now, I can say that IFSM has been registered as a not-for-profit organization in the state of Illinois, with a tax ID and consequently a legal entity, internationally;

(ii) select the site for IMC 18 to be held in 2014. You are all warmly invited to consider seriously this point. The best bid coming from any country should be selected out of the votes of the general assembly delegates. Please feel free to consult around you and to elaborate the most exciting and challenging proposal. There is no a priori barrier to your application. The detailed conditions (deadline) and required documents (constitution and documents to be included in your bid) will be detailed shortly in a mail from our General Secretary;

(iii) elect the new members of the IFSM Executive Committee to be in position from 2011 to 2014. Candidates for the President, for the General Secretary and for Committee members are now called for. The only position which will be automatically filled is that of Vice President which I will have the honour and pleasure to fill. Please prepare now your nominations for the new open positions;

(iv) the number of votes for your country in any election during the General Assembly, will be sent to you by the General Secretary. If you want to change your number of voting rights, you should apply during the general assembly with supporting arguments. The results will only apply during the 2014 general assembly.

Before closing this mail, I want to renew my invitation to let us know as soon as possible the changes which have occurred in your society (names of President, Vice President, Secretary, Treasurer, addresses and contacts, etc). We are also very keen to learn about the events which you plan to organize in 2010 and eventually in 2011, so that we can post them on our website. We already know that the regional meetings in 2012 will take place in London (EMC conference, see http://www.eurmicsoc.org/) and in Perth (APMC 10 conference).

2

During the past three years, I have attended myself and represented IFSM in a number of national and regional conferences, which has given me nice opportunities to meet many of you and your colleagues. I have enjoyed them very much, for sure. During the regional conferences in year 2008, the Executive Committee has met twice, in Aachen at EMC 2008 and in Jeju Island in Korea at APMC 9. Both meetings have been essentially devoted to the preparation of IMC 17 at Rio. I have also attended last September ICAM 2009 (an international conference in advanced materials) which took place in the same venue at Barra da Tijuca, where we will gather next September. This ICAM meeting, with about 800 participants, has been an ideal rehearsal for testing the facility and feeding back the IMC17 local organizers with a set of recommendations aiming at optimizing your welcome.

Waiting for the pleasure of meeting all of you soon in Rio de Janeiro

Sincerely

Christian Colliex

Preparing the future of IFSM, President, Vice President and General Secretary at the top
(photography recorded by Guillermo Solorzano at Cusco during the 2007 CIASEM conference)

Eventi internazionali

Eventi internazionali

Deutsche Gesellschaft für Elektronenmikroskopie e.V.

Deutsche Gesellschaft für Elektronenmikroskopie e.V.

(German Society for Electron Microscopy)

announces the

ERNST RUSKA PRIZE 2011

for outstanding achievements in the field of electron microscopy.

The Deutsche Gesellschaft für Elektronenmikroskopie invites to propose candidates for the Ernst-Ruska-Prize. The prize is awarded for work carried out by younger scientists pioneering new capabilities of electron microscopy as a scientific technique through innovative instrumentation or novel methods of basic and general interest. Work carried out by pure application of existing techniques will not be considered. The eligible work should not date back more than 7 years. It must be published or it must be accepted for publication at the time of submission of the proposal.

The decision will be made by an independent committee. The Ernst-Ruska-Prize consists of a certificate, a financial award, as well as the honor of giving an *Ernst-Ruska Distinguished Lecture* at the Ceremony of Award. If a group of authors receives the award, they will be awarded jointly. The ceremony will take place at the Microscopy Conference 2011 in Kiel, Germany, Aug. 28th-Sept. 2nd, 2011.

Proposals including appraisal of the achievement, reprints or preprints, and short CV including list of publications of the authors should be received (on paper and CD) not later than November 30th, 2010, addressed to

President of Deutsche Gesellschaft für Elektronenmikroskopie
PD Dr. Reinhard Rachel
Zentrum für EM der NWF III am Institut für Anatomie
Universität Regensburg
D-93053 Regensburg
GERMANY
E-mail: reinhard.rachel@biologie.uni-regensburg.de

Eventi internazionali

URBINO MCM 2011

10th Multinational Congress on Microscopy 2011

September 4 – 9, 2011

Scientific Campus Urbino University "Carlo Bo", Italy

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

President:
Elisabetta Falcieri
Department of Human, Environmental and Natural Sciences, Urbino, Italy
elisabetta.falcieri@mcm2011urbino.it

In the occasion of 10th anniversary of Multinational Congress, MCM2011 will be again organized by the Italian Society of Microscopical Sciences. Urbino, a middle-age city in the centre of Italy, with a big and ancient University, a pleasant climate and a beautiful nature, will be the congress site. The scientific program, all running in the University Scientific Campus, will comprise plenary lectures, symposia, poster sessions and workshops. A high quality trade exhibition will be also carefully organized in the wide Campus spaces. A Satellite Meeting on "Tomography and 3D reconstruction" will be organized on September 3 and 4 in Ancona, a lovely sea-side university city about 100 Km far from Urbino, and transfer to the congress venue will be planned by the Organization. Young scientist participation will be encouraged by offering fellowships, low conference fees and poster prizes.

ORGANIZING SECRETARIAT
italymeeting
info@italymeeting.it
www.italymeeting.it

www.mcm2011urbino.it

Functional nanostructures and interfaces of strontium ruthenates $Sr_3Ru_2O_7/Sr_2RuO_4$ eutectic crystals

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Vincitrice del Premio Carla Milanesi 2009

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Summary
Ruddlesden Popper $Sr_{n}+1Ru_nO_{3n+1}$ ruthenates exhibit a wide variety of exceptional properties providing an ideal playground to study new forms of ordering effects such as superconductivity in Sr_2RuO_4. A new normal metal $Sr_3Ru_2O_7$/superconducting Sr_2RuO_4 eutectic system has been successfully grown. Transport and magnetic studies on the eutectic material revealed the presence of an unexpected superconducting behaviour in the $Sr_3Ru_2O_7$ domain. Several scenarios have been proposed to explain this unusual behaviour addressing the crucial need of a detailed investigation by local probe techniques. We report the nanostructural characterisation of the $Sr_3Ru_2O_7/Sr_2RuO_4$ eutectic crystals by transmission electron microscopy and high angle annular dark field scanning transmission electron microscopy. We compared the nanostructure of the $Sr_3Ru_2O_7$ region of the eutectic with that of $Sr_3Ru_2O_7$ single-phase crystals in which no superconducting behaviour has ever been observed. We found that $Sr_3Ru_2O_7$ can be grown with higher purity via eutectic solidification showing only a diluted amount of randomly dispersed Sr_2RuO_4 layers. We also explored the $Sr_3Ru_2O_7/Sr_2RuO_4$ interfacial nanostructure. Two typologies of interfaces are observed within the eutectic: interfaces parallel to the growth direction are sharp and defect-free whereas interfaces perpendicular to the growth direction appear wavy and decorated with Ru precipitates. An exotic pairing between the Sr_2RuO_4 layers finely dispersed in the $Sr_3Ru_2O_7$ matrix is suggested as possible scenario for the unusual superconductivity of the $Sr_3Ru_2O_7$ domain of the eutectic.

Key words: Ruthenates, eutectic structure, HRTEM, HAADF.

Introduction

One of the most exciting developments of modern condensed-matter physics has been the discovery of new forms of order in materials with strong electron-electron interactions. The past decade has seen the observation of a whole range of novel superconducting, magnetic and metallic states. In many cases these new states are subtle and fragile, which means that they intrinsic properties can often only be observed in extremely pure crystals. Under this respect, a great role is held by the Ruddlesden-Popper (*R-P*) series of $Sr_{n+1}Ru_nO_{3n+1}$ strontium ruthenates, fascinating example of nature's engineer layered systems whose properties can be dramatically tuned by impurities and disorder. The typical structure of this class of compounds (see Figure 1) is made up of n consecutive perovskite layers ($SrRuO_3$) alternating with rocksalt layers (SrO) so that the formula can be written as $(SrO)(SrRuO_3)_n$. Since the discovery, nearly 40 years ago, of the distorted perovskite $SrRuO_3$ as a ferromagnetic metal with T_{curie}~160 K (Allen *et al.*, 1996), many studies of the R-P compounds have provided clear evidence

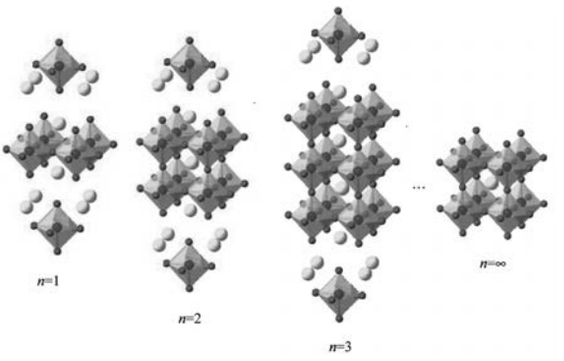

Figure 1. Crystal structure of the Ruddlesden–Popper series of $Sr_n+1Ru_nO_{3n}+1$ with n=1, 2, 3, and ∞.

that one of the most striking features of these oxides is that they display varying functionalities as the number of Ru-O layers per unit cell, n, increases (Cao *et al.*, 1999). Besides the unconventional spin triplet superconductor Sr_2RuO_4 (n=1) (Mackenzie and Maeno, 2003), the *R-P* series also includes the n=2 bilayered member, $Sr_3Ru_2O_7$ (Perry *et al.*, 2004), which is a metamagnetic normal metal with a quantum critical point, the nearly ferromagnetic $Sr_4Ru_3O_{10}$ (n=3) (Crawford *et al.*, 2002; Cao *et al.*, 2003b) and the itinerant ferromagnet $SrRuO_3$ (n=∞) (Mazin and Singh, 1997). In general, all the strontium ruthenates are metallic when they are grown in their stoichiometric forms and inclined to be magnetically ordered but small changes in either the crystal structure or the phase purity can switch their ground states all the way from correlated electron insulators to high-conductivity metals (Mackenzie and Y. Maeno, 2003; Nakatsuji and Y. Maeno, 2000; Grigera *et al.*, 2004). An outstanding example is the unconventional superconductivity observed in Sr_2RuO_4. This material, which is a close structural relative of high-temperature superconducting cuprates, is the first perovskite oxide superconductor with no copper. In contrast to most conventional superconductors which are largely immune to scattering from impurities or imperfections, the suppression of the superconducting transition temperature by non magnetic and magnetic impurities in this compound is one of the key signatures of its unconventional or non-s-wave superconductivity (Mackenzie *et al.*, 1998). The achievement of high quality samples is therefore a necessary prerequisite for the new superconducting state in Sr_2RuO_4 to be observed in the first place. Over the years, active investigations have been carried out to establish unambiguously the spin triplet superconductivity of Sr_2RuO_4. One of the major obstacle for understanding the physical properties of this ruthenate has been the lack of superconducting thin films. Tunnel experiments performed with superconductor-insulator-superconductor and superconductor-insulator-normal metal junctions based on Sr_2RuO_4 single crystals raised the crucial problem of controlling interfaces. The development of eutectic crystal growth of these systems has been proved to be an excellent route to merge together in a single composite crystal individual constituents with distinct physical properties thus offering the possibility to engineer new heterostructures with *ad hoc* functionalities. $Sr_3Ru_2O_7/Sr_2RuO_4$ eutectic crystals have been successfully grown by flux feeding floating zone technique (Fittipaldi *et al.*, 2004; Fittipaldi et al, 2008; Ciancio *et al.*, 2009). Such a composite structure, consisting of significant volume fractions of both Sr_2RuO_4 and the $Sr_3Ru_2O_7$, provides natural junctions and allows the study of tunnelling and proximity effect between the spin-triplet superconductor Sr_2RuO_4 and the nearly ferromagnetic metal $Sr_3Ru_2O_7$. The choice of $Sr_3Ru_2O_7$ as normal metal was driven by the high similarities in both the structural properties and growth conditions with the homologue Sr_2RuO_4: high purity single crystals of $Sr_3Ru_2O_7$ with a low residual resistivity comparable to that of Sr_2RuO_4 have been obtained. Such features represent a chance of a good crystalline matching at the interfaces with the possibility of good transport properties through the interfaces. Moreover it is worth to note that Sr_3RuO_7 is a really interesting compound in itself. $Sr_3Ru_2O_7$ was first found to be an anti-ferromagnetic metal (Cava *et al.*, 1997), then claimed as an enhanced paramagnetic metal with a changeover from paramagnetism to ferromagnetism under uniaxal pressure applied along its *c*-axis (Ikeda *et al.*, 2000). Lately $Sr_3Ru_2O_7$ has been reported as an itinerant metamagnet exhibiting quantum critical phenomena (Perry *et al.*, 2004). It is remarkable that no sign of superconductivity have been ever found even in the purest single crystals of $Sr_3Ru_2O_7$. During the course of the investigation of the functional properties of the $Sr_3Ru_2O_7/Sr_2RuO_4$ eutectics, an unexpected superconducting behaviour has been measured in the $Sr_3Ru_2O_7$ domain (Hooper *et al.*, 2006;

Fittipaldi *et al.*, 2008; Kittaka *et al.*, 2008). Several pictures have been proposed to explain this unusual behaviour such as a proximity effect (Hooper *et al.*, 2006) or, in analogy with the Ru-Sr_2RuO_4 eutectic system (Maeno *et al.*, 1998; Yaguchi *et al.*, 2003; Sigrist and Monien, 2001), the possible presence of an additional phase at the $Sr_3Ru_2O_7$ /Sr_2RuO_4 interfaces (Kittaka *et al.*, 2008). In this scenario, the investigation of the nanostructure of the $Sr_3Ru_2O_7$ domain of the Sr_2RuO_4- $Sr_3Ru_2O_7$ eutectic crystals is of crucial importance to extract the intrinsic properties of this ruthenate. This can be done by strictly comparing the nanostructure of the $Sr_3Ru_2O_7$ region of the eutectic with that of $Sr_3Ru_2O_7$ when it is grown as single phase crystal. Moreover, to verify weather the unexpected superconducting behaviour measured in the $Sr_3Ru_2O_7$ domain might be induced by the presence of an additional phase forming at the $Sr_3Ru_2O_7$ /Sr_2RuO_4 interfaces, it is crucial to determine atomic structure of the $Sr_3Ru_2O_7$ /Sr_2RuO_4 interfaces.

In this work, we provide a full characterisation of the nanostructures of the $Sr_3Ru_2O_7$/Sr_2RuO_4 eutectic crystals by transmission electron microscopy (TEM) and high angle annular dark field (HAADF) scanning TEM (STEM). A special emphasis is paid on the $Sr_3Ru_2O_7$ domain which is compared to $Sr_3Ru_2O_7$ grown as single phase material. A detailed characterisation of the atomic structure of the $Sr_3Ru_2O_7$/Sr_2RuO_4 interfaces is also provided.

Materials and Methods

The $Sr_3Ru_2O_7$/Sr_2RuO_4 eutectic crystals and the $Sr_3Ru_2O_7$ single phase crystals have been grown by flux feeding floating-zone technique with Ru self-flux, using a commercial image furnace equipped with double-elliptical mirrors and two 2.0 kW halogen lamps (NEC Machinery, model SC-K15HD-HP). An off-stoichiometric ratio k_{single}=1.6 and $k_{eutectic}$=1.45, respectively (where k=2N(Ru)/N(Sr), N(Ru) and N(Sr) being the mol fraction for Sr and Ru) was used to compensate the Ru evaporation during the crystal growth (R. Fittipaldi *et al.*, 2004). During the eutectic solidification, the $Sr_3Ru_2O_7$ and Sr_2RuO_4 phases solidify along the b direction in an ordered pattern consisting of alternating lamellae of thickness ranging from 60 to 200 μm piled up along the c direction. X-ray analysis showed only the presence of the Sr_2RuO_4 and $Sr_3Ru_2O_7$ phases which are fully aligned in the crystallographic directions (R. Fittipaldi *et al.*, 2004). Rietveld analysis of powdered eutectic crystals confirmed the previously determined ratio between the two phases. Although $Sr_3Ru_2O_7$ belongs to the orthorhombic space group Bbcb (Huang *et al.*, 1998; Shaked *et al.*, 2000), both Sr_2RuO_4 and $Sr_3Ru_2O_7$ can be considered as having a tetragonal crystal structure a=0.3862 nm and c=1.2722 nm, and a=0.38872 nm and c=2.0732 nm, respectively (Huang *et al.*, 1998; Chmaissem *et al.*,1998).

The TEM studies were performed using a Philips CM 200 field emission gun TEM with an attached Link ISIS EDS system and a Gatan Imaging Filter. HAADF analysis was carried out using a Titan 80-300 TEM/STEM with a probe spherical aberration corrector. Both instruments were operated at 200 kV. The HAADF images were recorded using a convergence semiangle of 24 mrad and a collection semiangle of 90 mrad giving Z contrast.

TEM specimens were extracted using a FEI Strata 235 DualBeam combined SEM and focused ion beam (FIB) instrument. 100-nm-thick TEM foils were thus obtained by a FIB-SEM liftout procedure and further thinned down to electron transparency by a Gatan Precision Ion Polishing System equipped with two Ar sources.

Results and Discussion

The image of Figure 2 taken by Polarized Light Optical Microscope (PLOM) gives an overview of the $Sr_3Ru_2O_7$/Sr_2RuO_4 lamellar pattern in the a-c plane. As further confirmed by Energy Dispersive Spectroscopy analysis carried out by Scanning Electron Microscopy (Ciancio *et al.*, 2009a; Ciancio *et al.*, 2009b), the dark contrast stripes are Sr_2RuO_4 lamellae embedded in the brighter contrast $Sr_3Ru_2O_7$ matrix. To explore the nanostructure of the $Sr_3Ru_2O_7$ domain of the eutectic thin membranes suitable for TEM analysis were extracted from the bulk crystals by the above mentioned lift-out technique. In Figure 3a a HRTEM micrograph taken in the [010] zone axis of the $Sr_3Ru_2O_7$ domain of the eutectic is displayed. As shown in the image, the crystal fragment has a well ordered nanostructure with no sizeable changes in the atom arrangement. The

typical atomic layer stacking of the $Sr_3Ru_2O_7$ phase constantly repeat all over the sample with a 2-nm-long periodicity corresponding to the *c*-axis value of the $Sr_3Ru_2O_7$ phase. Occasionally in the $Sr_3Ru_2O_7$ matrix layered defects identified by HAADF-STEM analysis as Sr_2RuO_4 layers (see Figure 3b) are observed which are distributed in a extremely small volume fraction and over distances of more than 50 nm from each other. This is confirmed by selected area electron diffraction (SAED) carried out over several regions of the sample. The SAED pattern in Figure 3a reveals only the characteristic spots of the $Sr_3Ru_2O_7$ phase thus confirming that the presence of Sr_2RuO_4 is very diluted to give discernable spots in the electron diffraction.

In contrast to $Sr_3Ru_2O_7$ grown via eutectic solidification, the nanostructure of the $Sr_3Ru_2O_7$ single phase crystal has a disorder typical of intergrowths. The HRTEM micrograph of Figure 4a taken with the electron beam parallel to the [010] direction, enlightens the presence of thin slabs (indicated by arrows) with different atomic stacking intercalating within the $Sr_3Ru_2O_7$ matrix. A diffuse dark contrast is generally associated with these areas as a result of the strain originating at the slab edges because of the different atomic stacking of the embedded region with the respect to the surrounding matrix. As better emphasised by Figure 4b, in which a zoom-in of the defective region is displayed, such phases are planar faults of $SrRuO_3$ embedded in the $Sr_3Ru_2O_7$ matrix. In addition, randomly distributed one unit-cell and half unit-cell-thick layers of $Sr_4Ru_3O_{10}$ are observed. The presence of such intergrowths is clearly visible in the SAED pattern in the inset of Figure 4a which reveals the presence of additional spots beyond the characteristic reflections of the double-layered $Sr_3Ru_2O_7$ structure, indexed as those of the orthorhombic $Sr_4Ru_3O_{10}$ in the [010]

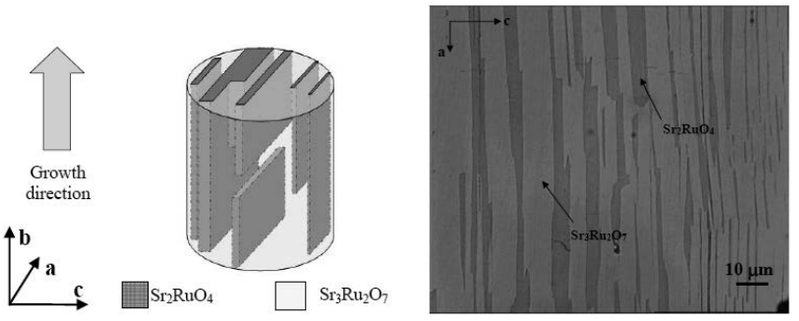

Figure 2. On the left a schematic drawing of the $Sr_2RuO_4/Sr_3Ru_2O_7$ eutectic crystal in which the three-dimensional lamellar structure is represented. On the right, an image taken by polarized light optical microscope of the $Sr_2RuO_4/Sr_3Ru_2O_7$ lamellar pattern in the *a-c* plane is shown (Ciancio *et al.*, 2009b, Ciancio *et al.*, 2010).

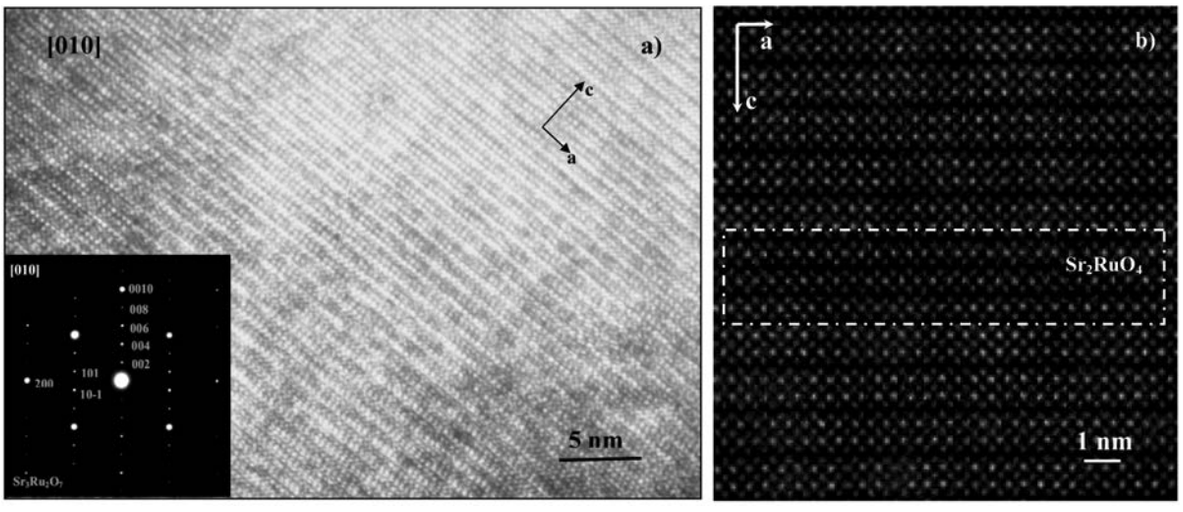

Figure 3. (a) HRTEM micrograph in the [010] zone axis showing the well-ordered atom arrangement of the $Sr_3Ru_2O_7$ region of the eutectic. (b) HAADF-STEM image showing the presence of Sr_2RuO_4 stacking faults (enlighten by the dash-edged box) in the $Sr_3Ru_2O_7$ matrix (Ciancio *et al.*, 2009a).

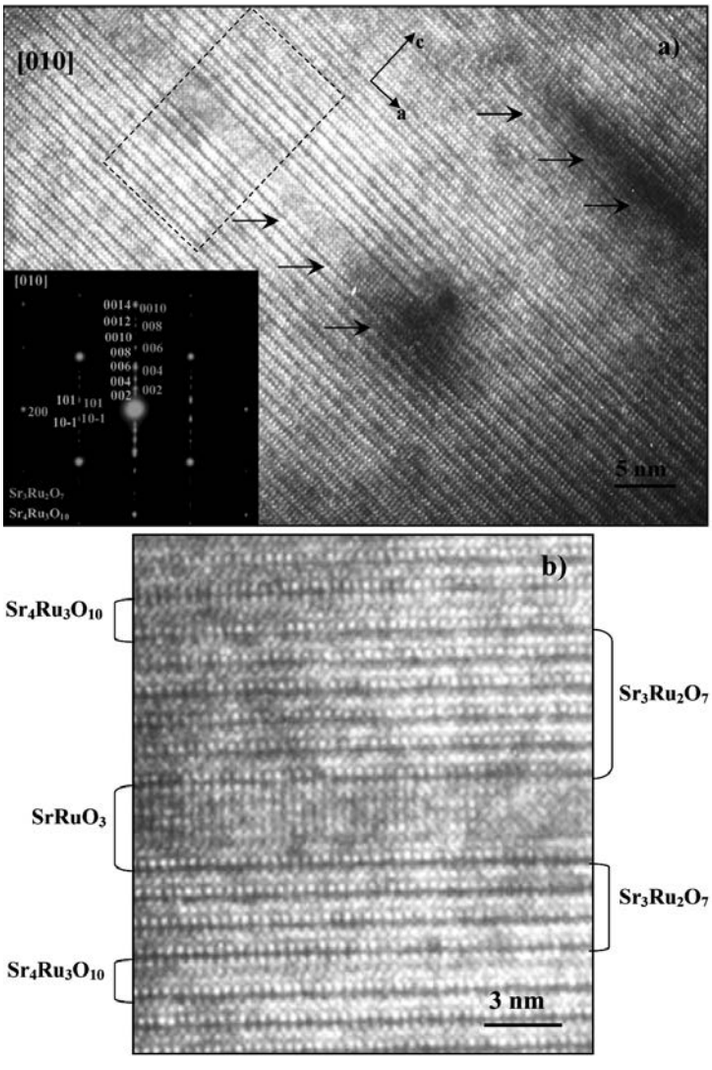

Figure 4. HRTEM micrographs of a $Sr_3Ru_2O_7$ single phase crystal taken in the [010] zone axis. In a) intergrowths of $SrRuO_3$ slabs intercalating within the $Sr_3Ru_2O_7$ matrix and associated with a diffuse dark contrast are indicated by arrows; in b) the defective region enlighten in a) by the dash-edged box is shown at higher magnification. A SrRuO3 slab and isolated half unit-cell of $Sr_4Ru_3O_{10}$ are displayed (Ciancio *et al.*, 2009a).

zone axis. The stronger intensity of the $Sr_3Ru_2O_7$ spots indicates a predominant contribution of $Sr_3Ru_2O_7$ ordered volume in the sample. In addition, diffuse streaks running through the fundamental spots are also seen, indicating a two-dimensional lattice defect on the *a*-*b* plane due to the presence of the intergrowths. The above remarks underscore the higher purity of the $Sr_3Ru_2O_7$ grown via eutectic solidification in which no atomically layered spurious phases are seen except for intergrowths of Sr_2RuO_4 occurring in very localized regions with poorly ordered volume to yield detectable spots in either XRD or electron diffraction. The statistics carried out on several representative regions showed that the total volume fraction of the Sr_2RuO_4 intergrowths is less than 5% of the imaged volume. The higher purity of the $Sr_3Ru_2O_7$ domain of the eutectic compared to $Sr_3Ru_2O_7$ single crystals, ascertained at atomic scale by TEM, is representative of the bulk samples, as confirmed by the magnetic measurements (Ciancio *et al.*, 2009) which reveal the presence of the magnetic transitions of the $SrRuO_3$ and $Sr_4Ru_3O_{10}$ phase.

The determination of the $Sr_3Ru_2O_7/Sr_2RuO_4$ interfacial nanostructure is another crucial point to be addressed to shed light on the unusual superconductivity measured in the eutectic. In particular, one of the crucial question to be answered is weather an additional phase might occur at the $Sr_3Ru_2O_7/Sr_2RuO_4$ interfaces thus justifying the insurgence of an additional superconductivity. As clearly shown in the PLOM image of Figure 2, the eutectic material provides two distinct typologies of interfaces namely along the *a* and *c* directions of the crystal. To explore the interfacial nanos-

tructure in the two different orientations, cross sectional samples were extracted by lift-out at the two interfacial regions and then analysed by TEM and HAADF-STEM (Ciancio *et al.*, 2009b). Our analysis revealed that the two interfacial regions have different nanostructures depending on their crystallographic orientation. As shown in the low magnification HRTEM micrographs of Figure 5, the interface parallel to the *a*-direction (Figure 5a) linearly proceeds over large distances and no elemental segregation can be seen across or in the vicinity of this region. However, the interface parallel to the *c*-axis (Figure 5b) is wavy over all the interfacial area with an amplitude of 50 nm. Defects and precipitates are distributed on either side of the interface over a 500 nm width region. The elemental segregation is higher on the Sr_2RuO_4 side appearing as a quite ordered distribution of nearly rectangular-shaped particles of dark contrast of a size ranging from 20 to 50 nm. EDS and HAADF analysis of the individual particles revealed that precipitates are Ru metal with hexagonal crystal structure. To completely rule out the possible presence of other phases at both $Sr_3Ru_2O_7$-Sr_2RuO_4 interfaces and to determine how the lattice interfacial match is built up, a more extensive characterisation was performed by STEM-HAADF. As shown in the HAADF image of Figure 6a taken in the [010] zone axis, the typical stacking of Sr_2RuO_4 (upper part) and $Sr_3Ru_2O_7$ (lower part) in the [010] direction can be discerned. The interface region is pointed by white arrows in the image. The brighter Ru columns are located in the center of the Sr–O columns with lower intensity. The matching at the interface between the two phases occurs with the sharing of the Sr–O rock salt, as shown in Figure 6b in which the white dash-edged region is shown aside the image at higher magnification with the corresponding labelling. The STEM-HAADF investigation was successful only in the case of the interfaces parallel to the *a*-axis. In the other case, the study was thwarted by the pronounced wiggling of the interface and by a consistent mismatch between the two phases. This can be seen by comparing the diffraction pattern taken in a defect-free area of the $Sr_3Ru_2O_7$ region in the [010] zone axis with that taken in a defect-free area of the Sr_2RuO_4 region keeping the tilt conditions of the $Sr_3Ru_2O_7$ region The SAED pattern corresponding to the Sr_2RuO_4 phase result to be out from his own [010] zone axis. Considering that the TEM sample

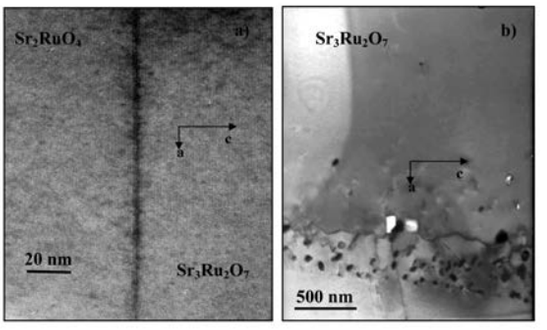

Figure 5. Overview of the two interfacial regions. In a) a TEM image taken in the [010] zone axis of the Sr_2RuO_4 /$Sr_3Ru_2O_7$ interfacial region with the interface along the *a* direction is shown. In b) a TEM micrograph in the [010] zone axis of the Sr_2RuO_4/$Sr_3Ru_2O_7$ interfacial region in which the wavy interface parallel to the *c* direction and the Ru precipitates (dark contrast particles) are seen (Ciancio *et al.*, 2009b).

holder allows the tilt of the specimen around two independent directions which are parallel to the in-plane crystallographic axis and a tilt of the specimen of 5° around the *a*-axis direction leads to the zone axis condition for the Sr_2RuO_4, the b axes of the two cells are tilted with respect to each other of about 5° around the *a*-axis direction. Such a misalignment only occurs in the *a*-*c* plane, where the two Sr_2RuO_4 and $Sr_3Ru_2O_7$ phase have to match along the *c*-axes which are different for the two cases. However, the lattice match along the *a*-axis is in an energetically favoured configuration because of the close values of the *a*-axis parameters of the two phases. As a consequence of such energy difference corresponding to the two interfacial orientations, it is understandable that elemental segregation only occurs at the interface parallel to the *c*-axis, where local change in chemical composition most likely can take place. These results are in a very good agreement with EBSD-SEM analysis performed on the bulk crystals (Ciancio *et al.*, 2010). In both cases no other phases are seen at the $Sr_3Ru_2O_7$/Sr_2RuO_4 interfaces thus ruling out the possibility of a superconductivity originated by the presence of additional phases at the $Sr_3Ru_2O_7$/Sr_2RuO_4 interfaces.

Conclusions

Eutectic solidification has been proved to be a fruitful way to grow high quality crystals. In particular, we show that $Sr_3Ru_2O_7$ grown via eutectic

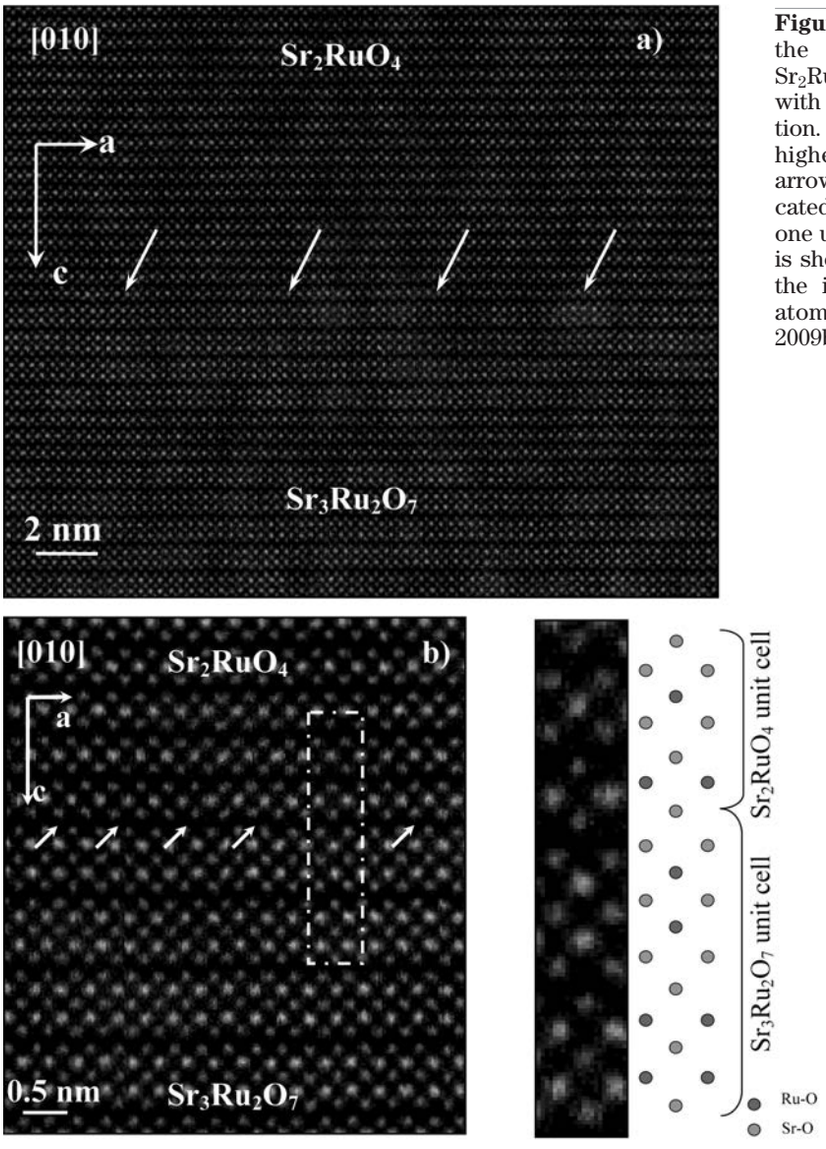

Figure 6. a) HAADF image taken in the [010] zone axis of the $Sr_2RuO_4/Sr_3Ru_2O_7$ interfacial region with the interface along the a direction. The interface region is shown at higher magnification in b) and arrowed. The area of the image indicated by the white rectangle, including one unit cell of Sr_2RuO_4 and $Sr_3Ru_2O_7$, is shown with higher resolution aside the image with a schematic of the atomic positions (Ciancio *et al.*, 2009b).

solidification has a significantly lower amount of layered spurious phase compared to $Sr_3Ru_2O_7$ single phase crystals, where intergrowths of $Sr_4Ru_3O_{10}$ and $SrRuO_3$ are found. The diluted presence of Sr_2RuO_4 stacking faults in the $Sr_3Ru_2O_7$ region of the eutectic excludes a simple percolation via proximity effect to justify the observed supercurrent and points on the possibility of the achievement of longer mean free paths in $Sr_3Ru_2O_7$ grown via eutectic solidification which would enable a long range communication via Kondo effect between the finely dispersed Sr_2RuO_4 layers. The analysis of the interfacial nanostructure in the three crystallographic orientations addressed crucial aspects on both the crystal growth and the potential application of this eutectic for the fabrication of spin-triplet tunnel junctions. Our studies revealed that the interfaces have different nanostructure depending on their orientation with respect to the growth direction of the crystal. The interfaces parallel to the both *a* direction are straight and defect-free due to the 'easy-axis' matching (the *a* axis parameters, are the same for Sr_2RuO_4 and $Sr_3Ru_2O_7$). On the contrary, along the 'hard-axis' matching (*c*-axis, which differs of about 0.8 nm in the two oxides), the interfaces are wiggly and decorated with Ru particles. Our investigations revealed that these last interfaces are the best candidates for tunnel experiments to study the order parameter symmetry of Sr_2RuO_4. The occurring of such elemental segrega-

tion in a specific crystallographic orientation opens the study of possible effects coming from the thermodynamic stability of this compound, such as the favourite energy surfaces, the phase formation dynamics, and the role of the active Ru evaporation during the growth. More in general, because of the high similarities between strontium ruthenates and other polymorphic oxides, these findings can be considered as representative of a wide class of materials and therefore applicable to the eutectic growth of other anisotropic oxide systems.

Acknowledgements

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Microvascular morphodynamics of swine periovulatory ovarian follicles as studied by SEM of vascular corrosion casts

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Summary

The growth of ovarian follicle and corpus luteum development is dependent on angiogenesis, the proliferation of new capillaries from pre-existing vessels. The morphological changes driven by angiogenesis ensure the adequate metabolic support to the follicular-luteal complex and are essential for fertility. However, the morphofunctional relation between neoangiogenesis and fertility are not yet fully understood, especially in the time between the LH surge and ovulation (periovulatory period). In this stage, somatic follicular cells rapidly differentiate in luteal steroidogenic cells. The metabolic switch, from an estrogen-to-steroidogenic activity, is sustained by extensive blood vessel remodelling. The purpose of this work was to describe the sequence of vascular remodelling events, rapidly occurring during the periovulatory period. Preovulatory, early periovulatory and late periovulatory follicles were obtained at a precise timing from prepubertal gilts stimulated by a validated hormonal protocol (eCG+hCG). The swine model was chosen due to a long periovulatory window (40-44 h, as in human). The three-dimensionality of the blood vessel network, the presence of angiogenesis and the patterns of angiogenic figures (sprouting or non-sprouting angiogenesis), were analysed by means of scanning electron microscopy of vascular corrosion casts. Results showed the presence of three concentric vascular plexuses (inner, medium and outer) in all groups. In the inner network, a high angiogenic activity was evidenced in preovulatory follicles, as demonstrated by a dense carpet of capillaries, with sprouting angiogenic figures. In early periovulatory follicles, the layer compactness decreased in consequence of an elongation of capillaries. The pattern of angiogenic figures was similar to the previous group. Close to the ovulation, in late periovulatory follicles, evident modifications in the vessel architecture were observed. The inner layer became undulated, as several folds were visible on its surface. Many gaps showed the characteristics of the underlying medium layer. Sprouting and non-sprouting angiogenesis, as intussusception, were visible. From the data obtained it is possible to conclude that the follicular angiogenesis in pig is high during the preovulatory phase, while the periovulatory period is constituted by a first step of quiescence followed by a second one of active angiogenesis, characterized by sprouting and, interestingly, non-sprouting angiogenesis. The quiescence status, in early periovulatory follicles, represents the starting point for the subsequent metamorphosing process, necessary to transform them into functional corpora lutea.

Key words: angiogenesis, vascular corrosion casts, scanning electron microscopy, ovarian follicles, pig.

Introduction

The formation of new blood vessels by migration and proliferation of pre-existing vessels is defined angiogenesis. In adults, vasculature is in a quiescent phase, with a turnover of endothelial cells lasting years. Physiological angiogenesis is prominent only in the female reproductive system, while the pathological one is related to tumour growth and metastatization (Plendl, 2000). In the ovary, new vessels are cyclically and rapidly formed during the reproductive lifespan due to angiogenesis in developing follicles and corpora lutea (Findlay, 1986; Reynolds *et al.*,

2002; Fraser, 2006). Angiogenesis is regularly followed or accompanied by a likewise rapid regression and disruption of the newly formed vessels, or angioregression, both in follicles and in corpora lutea (Suzuki *et al.*, 1998; Plendl, 2000; Tamanini and De Ambrogi, 2004). The cyclic growth and regression is regulated by a cyclic and pulsatile secretion of gonadotropins, which leads to ovulation and steroidogenesis (Geva and Jaffe, 2000; Motta *et al.*, 2003). The angiogenic waves involved in this phenomena are coordinated by extra-ovarian gonadotropins, as demonstrated in vivo and in vitro for LH (Luteinizing Hormone), FSH (Follicle Stimulating Hormone) and hCG (human Chorionic Gonadotropin) (Reisinger et al. 2007), and/or locally produced steroids (Rubanyi *et al.*, 2002). These morpho-functional changes, mainly occurring in the ovarian microvasculature domain, are essential to guarantee the adequate metabolic supply to the so called follicular-luteal complex (FLC). Moreover, since they appear to play important roles in folliculogenesis, ovarian hormone production and ovulation, it is evident the essential role of the angiogenic process to ensure fertility (Nottola *et al.*, 1997; Plendl, 2000; Macchiarelli, 2000; Motta *et al.*, 2003; Macchiarelli *et al.*, 2006; Jiang *et al.*, 2003, 2008).

The process of vascular remodelling in the FLC is particularly intense in the periovulatory period, when the ovulated follicle should rapidly differentiate into a highly vascularised structure: the corpus luteum. This structure, capable to synthesize high level of progesterone necessary to maintain the pregnancy, requires the support of an extensive blood vessel network, in order to ensure an adequate trophic supply to follicular/luteal cells (Hunter *et al.*, 2004). The rapid development of the ovulatory follicle in the subsequent curpus luteum is, indeed, sustained by an abundant but finely tuned angiogenesis. However, in this so peculiar moment, the sequence of angiogenic events, determined by specific morphological patterns of angiogenic figures (sprouting or non-sprouting angiogenesis), are not well known. Based on the above consideration, it becomes extremely interesting, from a morpho-functional point of view, to better understand the dynamics of blood vessels remodelling involved in the rapid events transforming the ovarian follicle into a corpus luteum. A better knowledge of such dynamics will be a valuable tool to discriminate among the factors of female infertility.

To this aim, the purpose of this work is to describe the sequence of vascular remodelling events, rapidly occurring in a well defined temporal moment: the periovulatory period, comprise between the LH surge and the ovulation of the oocyte.

Since angiogenesis is a phenomenon not only time-regulated but evolving in a three-dimensional pattern in space, to finely evaluate the morphological dynamics of the vascular network, the best technical approach is represented by Scanning Electron Microscopy applied to Vascular Corrosion Cast (SEM of VCC).

Materials and Methods

Experimental model

The polyovular swine model has been chosen because of the presence of: 1) multiple developing follicles and subsequent corpora lutea, allowing to follow the angiogenic dynamics in several follicular-luteal complexes; 2) a long periovulatory period (lasting 40–44 h, as in woman) which facilitates the analysis of the temporal evolution of angiogenesis and 3) a validated hormonal protocol, ensuring a precise estrous synchronization.

Since nowhere the scientific background about ovarian angiogenesis in pig has mainly involved normal cycling animals, the application of a validated hormonal protocol allowed an accurate selection of follicles at different moments of development. This was also helped by the presence of a long periovulatory window.

Ovarian collection

About fifteen prebubertal Large White gilt of approximately 100 Kg were injected i.m. with a single dose of 1250 IU of eCG (Folligon; Intervet) to promote follicular growth in 60-72 h (Shimizu *et al.*, 2002). After 60 h animals were injected with 750 IU of hCG (Corulon; Intervet) to induce ovulation (Martelli *et al.*, 2006; 2009). On the basis of the hormonal protocol, the treated animals were divided in three groups of five animals each, in order to obtain: a) a control group of preovulatory follicles (60 h after eCG administration), b) early periovulatory follicles (18 h after hCG administration) and c) late periovulatory follicles (36 h after hCG administration).

Ovaries were recovered by laparatomy from anaesthetized animals by an injection of azaperone (6 mL/gilt) and atropine sodium salt (2 mg/gilt) and maintained under thiopenthal sodium (1.5 g/gilt). All protocols had prior approval from the Ethical Committee of the University of Teramo.

Vascular corrosion casts and scanning electron microscopy

The ovaries of each animal were processed for the vascular corrosion casts (Jiang *et al.*, 2002) to obtain almost three-dimensional images at relatively high resolution (Murakami, 1971; Lametschwandtner *et al.*, 1990; Macchiarelli, 2000) and to highlight differences in the vascularization of the FLC, both in terms of angiogenesis that angioregression, depending on the stage of ovarian cycle. Moreover, the application of this technique allowed the evaluation of index of preservation, growth and regression of follicular cells, as the number of blood layers, shape and arrangement. In brief, to wash out all blood, swine ovaries were cannulated and perfused with a heparinised saline solution. All samples were, then, slowly perfused through the ovarian artery by a solution of Mercox, until polymerization started (Murakami *et al.*, 1971). The resin-injected ovaries were placed for 3-4 h in a warm water bath to complete polymerization, corroded in a NaOH (10%) solution for 24-48 h al 60°C, and gently washed for a few hours under tap water. Then, they were immersed in distilled water for 2-3 days at 60° C to completely remove macerated tissues and washed again under running tap water. Ovarian vascular casts were air dried, mounted onto aluminium stubs and coated with platinum. SEM observation were performed at low accelerating voltage (3-12 kV).

To examine the inner wall of follicular vessels, samples were frozen at -20°C, and then cracked with a razor blade. After immersion in alcohol (100%), samples were dried at 60°C overnight before further coating. The distinction between arteries and veins was made on arteries morphology (tortuous configuration and fusiform depressions on the surface of the casts due to endothelial cells prints) and veins morphology (straight configuration with rounded shallow endothelial depressions). The capillary network extension was evaluated qualitatively (Macchiarelli *et al.*, 1991; Nottola *et al.*, 1997; Jiang *et al.*, 2002; 2003) and quantitatively according to morphometric methods (Lametschwandtner *et al.*, 1990; Minnich *et al.*, 2001).

Results

General vascular architecture of swine ovarian follicles

SEM observation of VCC allowed the identification of numerous vascular plexuses, in the follicular architecture, mainly of ovoid shape and with different size, that appeared well-perfused by the casting medium. Vessels were classified according to their diameters and shapes of endothelial cell nuclei (Macchiarelli *et al.*, 1991, 1992, 1993, 1995, 1998). Indeed, arteries showed a tortuous configuration and presented fusiform endothelial cell print depression on the surface of the casts, while veins showed a rather straight course and possessed rounded depressions (Takada *et al.*, 1987). Characteristic figures of follicular angiogenesis (i.e. budding, growth and division of capillaries from pre-existing blood vessels) were easily distinguishable in all the samples (Macchiarelli *et al.*, 2006).

In detail, in all the groups analysed, ovarian follicles showed three concentric vascular plexuses connected to each other by anastomotical bridges: an inner, a medium and an outer vascular network (Figure 1A, 1B, 1C). Since the microvascular architecture presented a cup-like aspect, with an empty central cavity (before corrosion occupied by the granulosa layer, oocyte and antrum cavity), in the inner layer it was possible to verify the presence of regional-specific differences in the distribution pattern of angiogenic figures. In fact, three area (apical, equatorial and basal) were identified in the casted ovarian follicles and the number of angiogenic structures were counted in each of them.

Group 1

In preovulatory follicles (control group) the inner network evidenced a peculiar carpet-like aspect, due to a very high density of short and small capillaries (~7 µm in diameter), presenting angiogenic figures such as budding and sprouting (Figure 2A). The number of angiogenic structures was significantly higher in the equatorial region (13.25 ± 1.45^{a}), respect to the basal (3.01±0.88) and apical (1.05±0.49) ones. The medium vascular

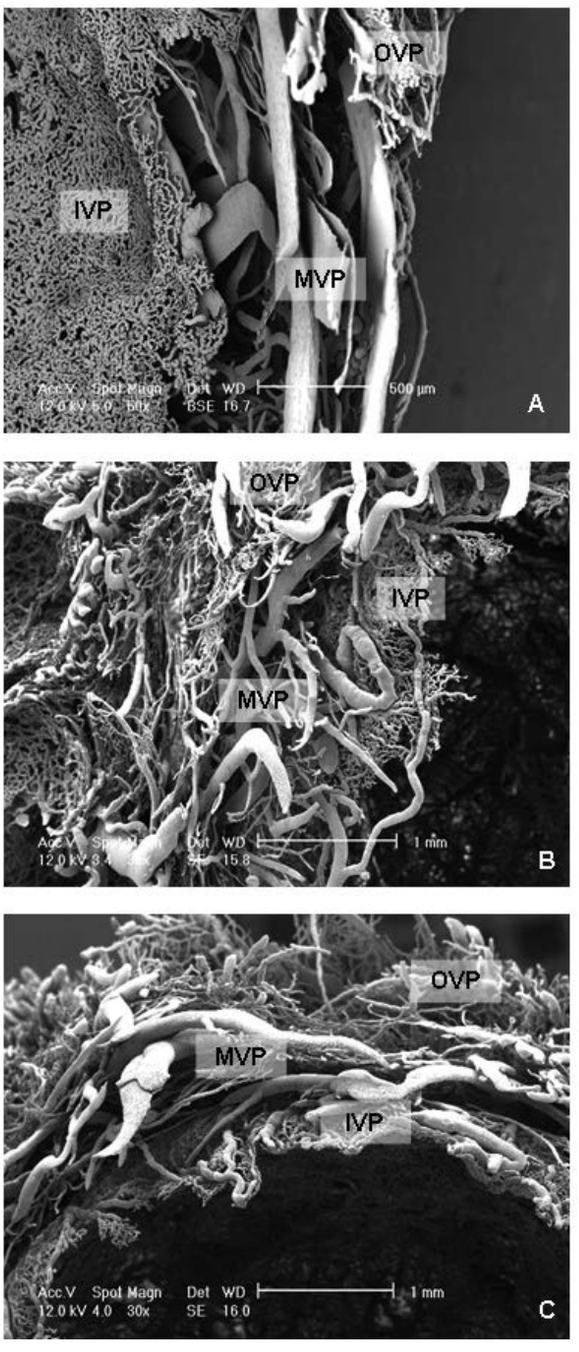

Figure 1. Representative pictures of the inner (IVS), medium (MVS) and outer (OVS) vascular plexus as observed in gilts by SEM of VCC at low magnification in preovulatory (A), early periovulatory (B) and late periovulatory ovarian follicles.

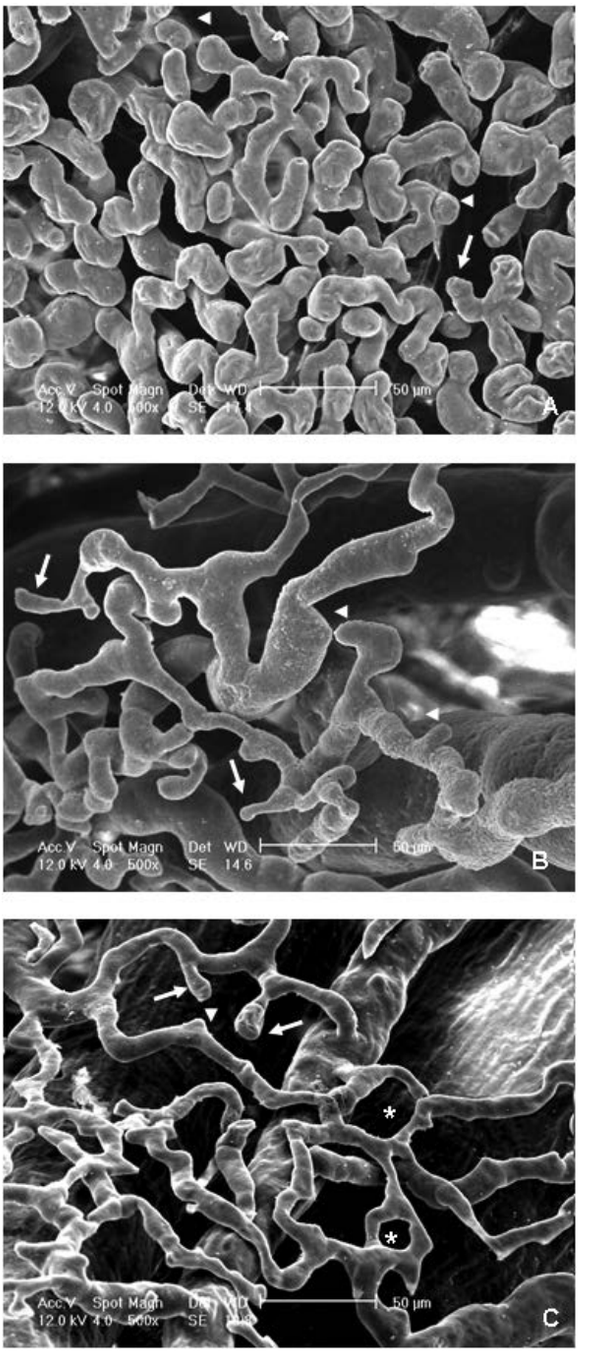

Figure 2. SEM of VCC micrographs at high magnification of the inner vascular plexus in preovulatory (A), early periovulatory (B) and late periovulatory pig ovarian follicles. Angiogenic figures are indicated: budding (arrowhead), elongation of capillaries (arrow) and intussusception (asterisk).

plexus was mainly constituted by large vessels, with a diameter of about 46 µm, necessary to support the inner plexus. The outer network was characterized by thinner vessels (~11 µm in diameter), distributed at different levels (Figure 1A).

Group 2

In early periovulatory follicles, the inner network showed comparable thickness and blood vessel diameter (~6 µm) to group 1. However, differently to preovulatory follicles, an evident elongation of capillaries, associated to a consequent decrease in the layer compactness, was observed. Budding and sprouting were the angiogenic figures present (Figure 2B); their number was not statistically different, respect to group 1, in the apical (1.05±0.49 vs 1.97±0.78) and basal (3.01±0.88 vs 4.02±1.01) regions of the inner layer. On the contrary, it was observed a statistically significant decrease in the equatorial area (13.25 ± 1.45^{a} vs 4.76 ± 0.99^{b}). In the medium layer, blood vessels resulted similar to its analogous in preovulatory follicles (44.40 vs 46.23 µm, respectively). Arterioles and venules from the middle plexus showed a low density, but they were visible underneath the gaps of the inner network. The vessels from the outer plexus showed similar characteristics to group 1 (Figure 1B).

Group 3

Close to the ovulation, the inner layer architecture in late periovulatory follicles was similar to groups 1 and 2, in terms of thickness and vascular diameters (~8 µm). However, differently to them, this layer showed an undulated aspect, with ridges, folds and numerous large gaps dispersed among. It was intriguing to understand that these phenomena of infolding, observed for the first time at this stage, were determined by the vessels from the middle network that lifted in folds the inner plexus. The inner layer displayed several angiogenic figures, differently distributed among the regions (the uncasted apical region was not determinable). Their number in the equatorial region, statistically decreased respect to group 2 (4.76 ± 0.99^{b} vs 11.45 ± 1.57^{a}, respectively), while did not show differences in the basal region (4.02±1.01 3.91±1.56). The figures were mainly constituted by sprouting and non-sprouting (as intussusceptions) angiogenesis (Figure 2C). In particular, the inner vascular plexus showed the formation of numerous meshes and transcapillary pillars raging from 3 to 20 µm. The intussusceptive pillar appeared shortly distant from the bifurcation or in the centre of the vessel in some circumstances, while in others holes appeared within the capillary bed, otherwise long parallel rows of pillars appeared in longitudinal folds of the endothelium. Due to the presence of several folds, the thickness of the middle layer was bigger, even if the vessel diameter was similar to the other groups (~ 65 µm). In the outer plexus, vessel diameter and distribution were similar to groups 1 and 2 (~ 11 µm) (Figure 1C).

Discussion

Angiogenesis is the physiological process by which new blood vessels originate from a preexisting microvasculature. It has a key role in embryonic development, adult organ growing, functioning and survival but especially in the reproductive physiology as the normal menstrual cycle and pregnancy (Robinson *et al.*, 2009). An active angiogenesis, however, accompanies also several pathological conditions such as tumour (Cuevas and Boundreau, 2009), inflammation (Jackson *et al.*, 1997), rheumatoid arthritis (Szekanecz *et al.*, 2009). The pathogenesis of numerous other diseases is also referable to insufficient growth and maturation of the vascular network (Ferrara et al, 2005). Despite in the last years scientific literature acquired many information on the morphological expression of angiogenesis in different experimental models as well as on its regulatory molecular mechanisms, this biological process is still only partially known.

The interest of our research group to unravel the morpho-functional mechanisms of angiogenesis in the female reproductive system is due to its important role exerted to guarantee a reproductive success. In fact, defects in ovarian angiogenesis may contribute to ovarian dysfunction and infertility. For example, in infertile women, decreased ovarian vascularity, consequence of a down-regulation of angiogenesis, has been associated with lower pregnancy rates. Polycystic ovarian syndrome (PCOS), ovarian hyperstimulation syndrome (OHSS) and ovarian cancer are pathologies associated, on the contrary, with an up-regulation of angiogenesis, inducing vessels

hyper-permeability (Geva and Jaffe, 2000).

From what reported above it is now clear that a correct sequence of follicular growth and development, accompanied by a perfectly balanced angiogenesis, can ensure the ovulation and, indeed, fertility.

The peculiar moment in the follicular development, studied here, is that immediately following the LH surge and ending with the ovulation: the so named "periovulatory period". During this phase, lasting 40-44 hours in pig, important spatio-temporal remodelling in the follicular microvascular architecture occurs. The ovarian follicle, a limited blood supplied structure, should transform in a well vascularized organ, the corpus luteum, to sustain the pregnancy.

The use of SEM applied on vascular corrosion casts, allowed to obtain quasi three-dimensional images at relatively high resolution (Murakami, 1971; Lametschwandtner *et al.*, 1990; Macchiarelli, 2000) and to highlight differences in the vascularization of the FLC, both in terms of angiogenesis that angioregression, depending on the stage of ovarian cycle.

Results obtained showed the extremely high angiogenic plasticity of the periovulatory follicle. The preovulatory period that precedes the LH surge (follicles from group 1) is characterized by a discrete angiogenic activity, as evidenced by the compact layer of capillaries in the inner network, presenting phenomena of sprouting angiogenesis, especially in the equatorial region. At the beginning of the periovulatory period (follicles from group 2) a quiescence in the angiogenesis is detected. In fact, respect to group 1, it was observed a decrease in the inner layer compactness, accompanied by a parallel elongation of capillaries, and in the number of angiogenic figures of the equatorial region. Approaching to the ovulation (follicles from group 3), an intense vascular remodelling restart. The vascular area of the inner vascular plexus increases so evidently, as sustained by sprouting and non-sprouting (as intussusception) angiogenesis, to refold into the antrum. The infolding was due to the action of vessels from the middle network. From what observed by SEM observation at higher magnification, the transcapillary pillars formation was not detected before the late periovulatory phase, suggesting that intussusception did not occur before this developmental stage. Differently to sprouting, the non-sprouting (intussusceptive) angiogenesis occurs almost in absence of endothelial cell proliferation, is achieved at low vascular permeability levels, and requires only 4-5 hours for completion (Djonov *et al.*, 2003). It it easy to argument that intussusception is the optimal physiological solution to sustain a rapid angiogenesis during the quick transformation from the ovulatory follicle into a functional corpus luteum (Djonov *et al.*, 2003; Burri *et al.*, 2004).

In conclusion, these data allow to hypothesize a metamorphosing nature of the early periovulatory follicle that, over a few hours, transforms into a highly vascularized structure, probably in order to sustain the corpus luteum development after ovulation.

Since angiogenesis is a phenomenon which evolves in a three-dimensional pattern, the application of SEM of VCC confirm to be the technique of choice to: i) determine the indexes of preservation, growth and regression of FLCs; ii) evidence the number, shape and arrangement of blood layers and iii) to clarify the patterns of angiogenic figure (sprouting and/or non-sprouting) present during the different stages of development in FLCs (Macchiarelli *et al.*, 2006).

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Melatonin prevents hydrogen peroxide-induced apoptotic cell death

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Summary

Melatonin (MEL) functions in organisms are diverse. The actions considered in the current work relate to its ability to prevent oxidative stress, i.e. molecular damage produced by reactive oxygen species (ROS). Apoptosis is an active form of cell death that is initiated by a variety of stimuli, some of which inducing ROS increase. Hydrogen peroxide (H_2O_2) is considered a typical cytotoxic and oxidant agent capable to induce cellular damage through free radical production. In the present work we have investigated its role in the induction of cell death. U937 cells were exposed to 500 µM H_2O_2 and MEL behaviour, in the presence of this type of oxidative stress, was evaluated by incubating cells with MEL before and after H_2O_2 exposure. Cytometric and microscopy analyses were utilized and revealed that H_2O_2 can be considered an apoptotic trigger, if used at proper concentrations. In fact U937 cells, after 500 µM H_2O_2 exposure, showed chromatin condensation, micronuclei presence, apoptotic bodies and secondary necrosis. MEL, added before H_2O_2 treatment, significantly reduced apoptotic cell number. On the contrary, MEL incubation after H_2O_2 induced an increase of apoptotic cell death, and cells in secondary necrosis were detectable. These results indicated that pre-incubation with MEL reduces H_2O_2-dependent apoptosis in U937 cells, suggesting a capacity of this hormone to interfere with apoptosis induced by ROS increase.

Key words: U937, melatonin, hydrogen peroxide, apoptosis, reactive oxygen species.

Introduction

Melatonin (N-acetyl-5-methoxytryptamine), the hormone produced by the pineal gland, was shown to have significantly broad actions including oncostatic effects (Bejarano *et al.*, 2009), immune system stimulation (Guerrero and Reiter, 2002) and anti-inflammatory functions (Radogna *et al.*, 2009). Subsequently, MEL was identified as a direct free radical scavenger (Reiter and Korkmaz, 2009) and an indirect antioxidant (Rodriguez *et al.*, 2004). Its function consists in the reduction of oxidative stress, i.e. molecular damage produced by reactive oxygen and nitrogen species (Reiter, 2008). Numerous reports evidenced MEL ability to neutralize free radicals, molecules that have an unpaired electron in their valence orbital, as hydroxyl (•OH) (Li *et al.*, 2002) and oxygen radical (O_2•$^-$). Melatonin is also a scavenger of hydrogen peroxide (Tan *et al.*, 2000), a non-radical ROS, generated *in vivo* by several enzyme systems and produced intracellularly by the dismutation of the superoxide anion radical (O_2•$^-$). In addition, it is known that MEL acts by indirectly stimulating the antioxidative enzymes, that represent a major defence mechanism against free radical damage.

Literature evidences that MEL, through both antioxidant and scavenger functions, plays an important role in preventing apoptosis: its anti-apoptotic activity was described in several systems, including cerebellar neurons (Baydas *et al.*, 2002) and brain astrocytes (Jou *et al.*, 2004), where it was hypothesized to have an antioxidant

role. A recent study demonstrated MEL capacity to prevent UVB-induced apoptosis in U937 cellular line (Luchetti *et al.*, 2006). In the present work, we have evaluated, by means of cytometric and morphological techniques, cell death induced by H_2O_2 treatment in the same cells, investigating, in particular, MEL function in preventing cell damage induced by ROS increase generated by H_2O_2 exposure.

Materials and Methods

Cell culture

U937 human myelomonocytic lymphoma cell line was grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 25 mM Hepes pH 7.5, 1% antibiotics and maintained at 37°C in humidified air with 5% CO_2. Cell viability was assessed by MTT test (Mosmann, 1983).

For the induction of apoptosis, cells (seeded at 1x106 cells/mL) were exposed to 500 µM H_2O_2 (Fukamachi *et al.*, 1998) for 3h at 37°C in humidified air with 5% CO_2.

MEL (Sigma) was first dissolved in absolute ethanol at initial 100 mM concentration and then diluted at final 1 mM concentration in culture medium. 1 mM MEL treatment (Luchetti *et al.*, 2006) was performed both immediately before and after H_2O_2 treatment; subsequently, the treated cells were post-incubated as described above.

Flow cytometry (FC)

Cell death features (early and late apoptotic, as well as necrotic cells) were evaluated using propidium iodide (PI) staining carried out according to manufacturer's instructions. For FACS analysis 1×10^6 cells were collected by centrifugation at 300 g for 5 min at 4°C and washed once in ice-cold PBS. Cells were fixed (and permeabilized) with -20°C cold 70% ethanol overnight at 4°C. Fixed cells were washed in PBS once and resuspended in 1 mL of staining solution (40 µg/mL Propidium Iodide and 100 µg/mL RNase A in PBS). The samples were incubated for 30 min at room temperature in the dark. All samples were analyzed by EPICS XL flow cytometer (Beckman Coulter, Miami, FL, USA) with the appropriate software (System II, Beckman Coulter). Data were analyzed and histograms were plotted using winMDI version 2.9 flow cytometry application software (Scripps Research Institute, La Jolla, CA, USA). Values of PI fluorescence were presented as DNA histograms, showing cell distribution into the main cycle phases (G0/1 =M1, S=M2, G2-M= M3) and where the possible presence of apoptotic cell death can be recognize by typical sub-G1 peak (M4).

Light and electron microscopy

Differently treated U937 pellets were immediately fixed in 2.5% glutaraldehyde in 0.1 M Sörensen phosphate buffer pH 7.3, postfixed in 1% OsO_4 in the same buffer, dehydrated with ethanol and embedded in araldite as previously described (Burattini *et al.*, 2009). Semithin sections were stained at 50-60°C with a mixture of 1% methylene blue and 1% toluidine blue in distilled water. Thin sections were collected on nickel grids, stained with uranyl acetate and lead citrate, and observed with a Philips CM10 electron microscope.

Results

MTT test (Figure 1) revealed a good cell vitality for control (100%) and MEL alone treated (95.4%) cells, but it decreased to 75% after H_2O_2. MEL added before H_2O_2 treatment reduced cell death and viability was 83.9%; on the contrary, MEL added after H_2O_2 exposure showed a behaviour similar to that with H_2O_2 alone, and cell vitality was 74%.

Flow cytometry (Figure 2) evidenced that both control (2A) and cells treated with MEL alone (2B) showed only 1.5% and 2.9%, apoptotic/necrotic cells respectively, revealing a good cell viability.

For both conditions a well preserved cell morphology was confirmed by reverse microscope (RM), semithin sections and ultrastructural analyses (Figure 3A, B, C, D; 4A, B).

PI staining showed that H_2O_2 treatment (Figure 2C) induced apoptosis, quantified as 10.6% of PI / FITC ANX-V positive cells. RM and LM showed a variety of apoptotic cells (Figure 3E, F). TEM observations, after H_2O_2 treatment, evidenced characteristic apoptotic patterns (Figure 4C), such as chromatin margination towards nuclear periphery with typical cup-shaped patches (Figure 4D). Micronuclei, scattered throughout the cytoplasm and progressively released in

extracellular space, characterize the late apoptotic stage (Figure 4E).

When cells were treated with 1mM MEL before H_2O_2, apoptotic cell number was similar to that of the control (Figure 2D). Its percentage appeared, in fact, strongly decreased (2.9%), as confirmed by RM, LM and TEM, that showed a low number of apoptotic cells (Figure 3G, H and 4F, G). Differently, MEL treatment after H_2O_2 exposure determined an apoptotic/necrotic cell increase, respect to H_2O_2 alone. In fact, percentages detected were 31% of PI/FITC ANX-V positive events (Figure 2E). A higher number of apoptotic cells could also be revealed at RM, LM and TEM, generally characterized by late apoptotic pattern and secondary necrosis (Figure 3I, L and 4H, I).

Discussion

MEL is an effective antioxidant molecule and a free radical scavenger. Its efficacy is also correlat-

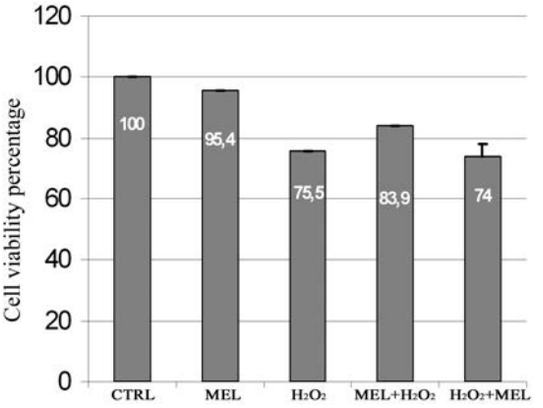

Figure 1. MTT assay test. A high percentage of cell vitality appears in both control and MEL treated cells (100% and 95.4% respectively). A reduction of cell viability (75.7%) was obtained after H_2O_2 treatment. When cells were pre-treated with 1 mM MEL their vitaility increases to 83.9%, whereas when MEL was added after H_2O_2 exposure a behaviour similar to H_2O_2 treatment (74%) was observed. Data were collected from three experimental conditions and they were expressed as mean +/- standard error.

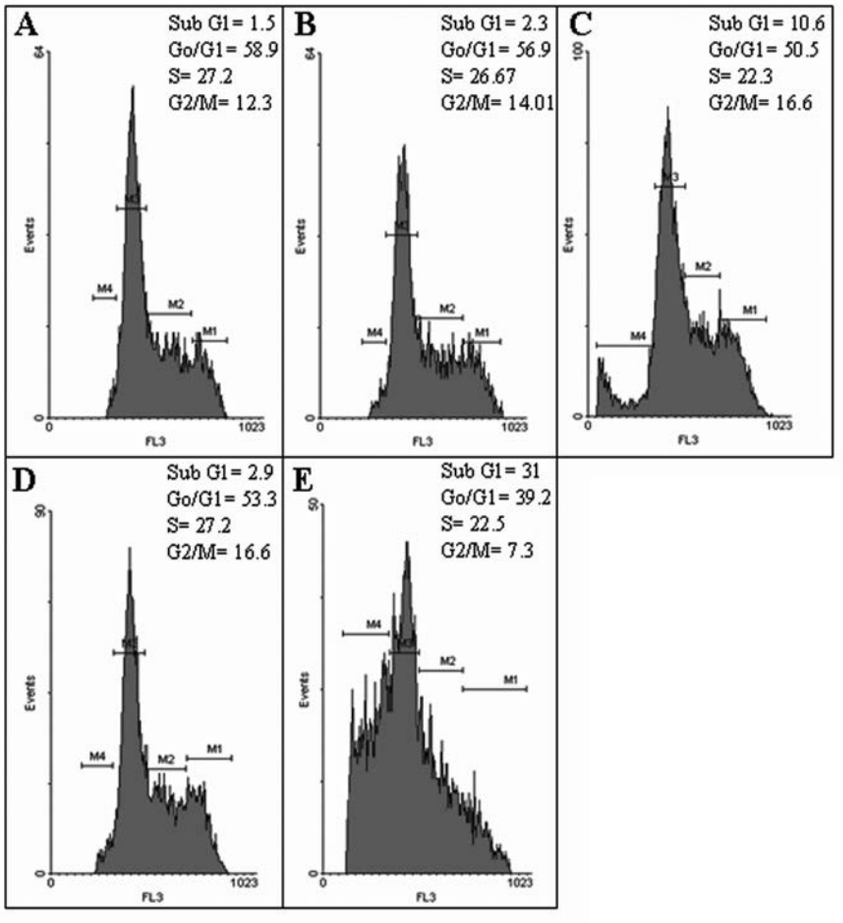

Figure 2. FC analysis of DNA content. U937 control cells and cells treated with 1mM MEL alone show a similar distribution of cell cycle phases (A and B, respectively). H_2O_2 treated cells reveal a weakly subdiploid peak (C), drastically reduced when cells were MEL-pretreated (D). On the contrary, an evident increase of subG1 events appears when U937 cells are incubated with 1 mM MEL after H_2O_2 exposure (E). Representative cytograms are taken from three independent experiments for all experimental conditions

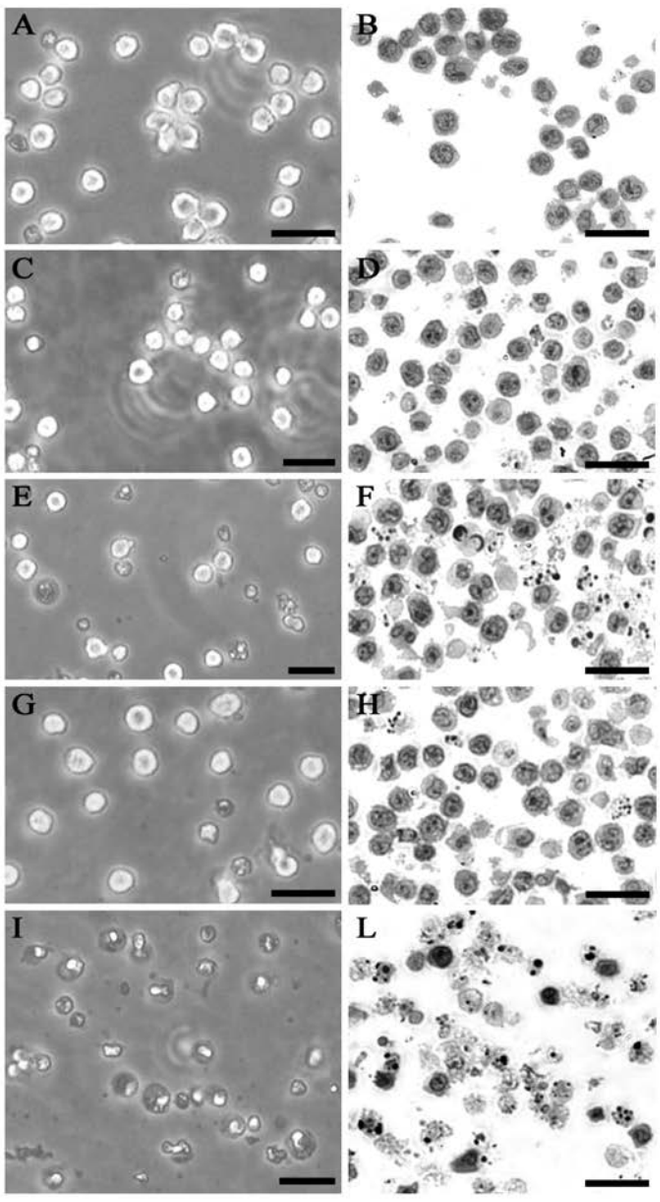

Figure 3. U937 cells at RM (A, C, E, G, I) and LM (B, D, F, H, L). Control cells (A, B) and MEL alone treated cells (C, D) show a well preserved morphology. After H_2O_2 treatment, apoptotic cell death is evident (E, F). When MEL is added before H_2O_2 treatment, only few cells appear apoptotic, being the others similar to the control (G, H). When MEL is added after H_2O_2 exposure several apoptotic cells are observed (I, L). Bar = 10 µm.

ed to ability to enhance the activities of a variety of antioxidative enzymes (Rodriguez *et al.*, 2004), stimulatory actions on glutathione synthesis (Winiarska *et al.*, 2006), reduction of electron leakage from the mitochondrial electron transport chain (Leon *et al.*, 2006), and to its synergic interactions with other antioxidants (Lopez-Burillo *et al.*, 2003).

MEL was originally shown to inactivate the highly toxic hydroxyl radical (•OH). Since this discovery, its scavenging repertoire has been demonstrated against H_2O_2 (Tan *et al.*, 2000), hypochlorous acid (HOCl) (Zavodnik *et al.*, 2004), singlet oxygen (O_2•) (Matuszak *et al.*, 2003), superoxide anion radical (O_2 •$^-$), nitric oxide (NO•) (Aydogan *et al.*, 2006), peroxynitrite anion ($ONOO^-$) (Reiter *et al.*, 2001) and others (Hardeland *et al.*, 2005).

In addition, it also favours the defence against oxidative stress by promoting enzymes that metabolize radicals and their products to innocuous agents (Reiter *et al.*, 2008). These indirect MEL antioxidative effects may be mediated *via* membrane and/or nuclear receptors. Examples

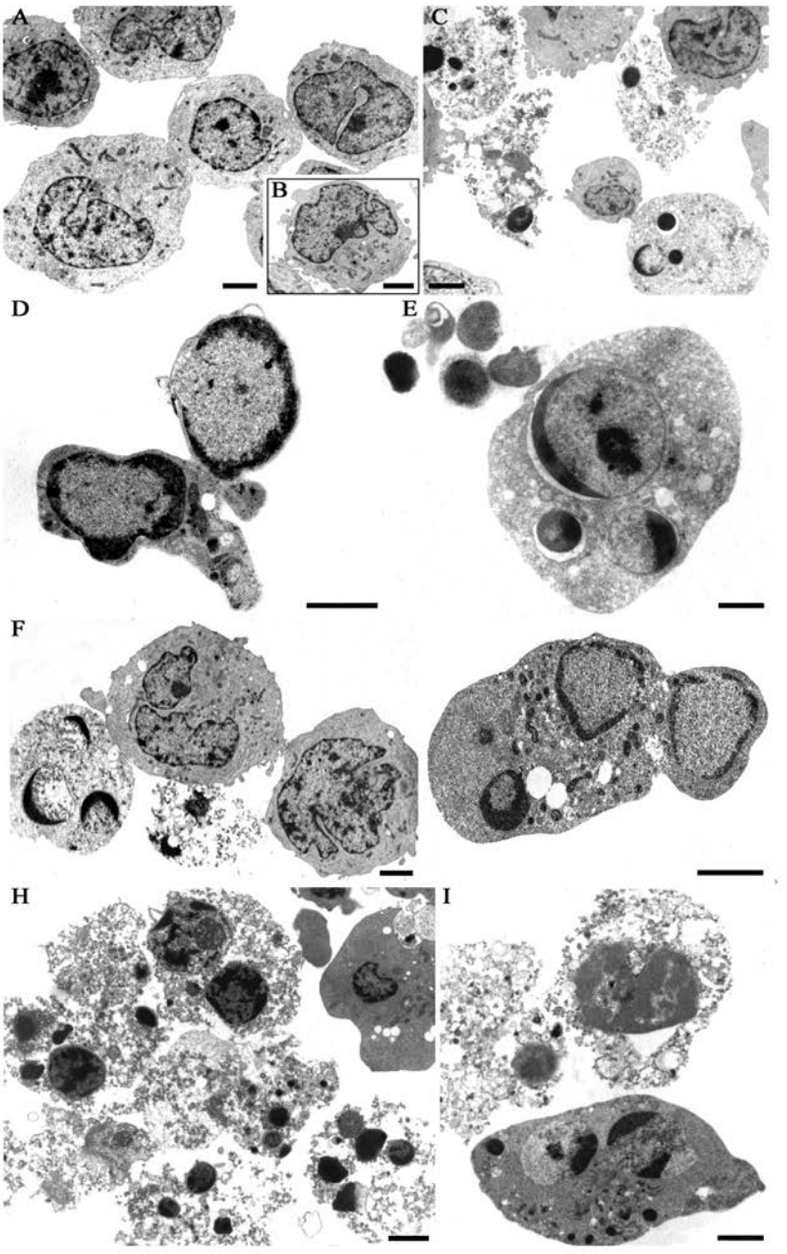

Figure 4. TEM analysis. Control cells (A) and U937 treated with MEL alone (B) show a comparable ultrastructural good morphology. After 500 µM H_2O_2 treatment, apoptosis appears in all apoptotic stages (C, D, E). Pre-treatment with MEL and successive exposure to H_2O_2 evidences a decrease of apoptotic cell number (F,G). When cells are exposed to H_2O_2 and then treated with MEL, apoptotic cell number appears strongly increased, with cells in late apoptotic stages, micronuclei, and secondary necrosis (H, I). A, C, F, H, bar = 2.5 µm. B, D, F, G, I bar = 2 µm.

include the widely reported stimulation of glutathione peroxidase (GPx) (Rodriguez *et al.*, 2004), which converts hydroperoxides, including H_2O_2, to water and oxygen, while oxidizing GSH.

In this study we have examined the effect of MEL on apoptosis induced via oxidative stress generated by H_2O_2. Our data show that H_2O_2, while being a free radical inducer, can be considered not only a cytotoxic agent, but an important apoptotic trigger, if utilized at proper concentration i.e. 500 µM. In addition, this study confirms MEL action as an effective antioxidant and free radical scavenger, with a possible protective role from apoptotic /oxidative damage.

These findings are in agreement with our previous study (Luchetti *et al.*, 2006), which reported that MEL was able to prevent UVB-induced apoptosis in U937 cells. Both UVB radiations and H_2O_2 lead to ROS generation and cell damage, but UVB seem a more powerful apoptotic trigger. To this

regard, a study on keratinocytes demonstrated that both UVB and H_2O_2 led to the increase of intracellular H_2O_2 levels, the antioxidants catalase and glutathione monoester, inhibited apoptosis only when induced by H_2O_2 and not by UVB (Chang *et al.*, 2003). In addition, DNA damage in the form of cyclobutane pyrimidine dimers was observed after exposure to UVB, but no photoproducts were found in H_2O_2-treated cells, suggesting a ROS-independent pathway of UVB-induced apoptosis (Chang *et al.*, 2003). In the present study apoptosis is a weak process if compared with that induced by UVB.

Neverthless, Luchetti *et al.*, (2006) showed that apoptosis was reduced when MEL is added both before and after UVB exposure. Differently, in the present study MEL prevents apoptosis only when added before H_2O_2. Differently, MEL incubation after H_2O_2 treatment lead to an increase of apoptotic cell death, suggesting that H_2O_2 intracellular level increases and free radical production can not be further controlled by MEL. A recent work on mouse mitochondria incubated with MEL, evidenced its capacity to down regulate oxygen consumption, to inhibit oxygen flux increase in the presence of an excess of ADP, to reduce membrane potential, and, consequently, to prevent superoxide anion and hydrogen peroxide production (Lopez *et al.*, 2009).

This study emphasizes MEL role as effective antioxidant and anti-apoptotic trigger. In fact, besides its direct scavenging actions, MEL stimulates several antioxidative enzymes, including superoxide dismutase, glutathione peroxidase and glutathione reductase (Reiter *et al.*, 2002). Therefore, in our model MEL pre-treatment prevents H_2O_2 induced apoptosis because of MEL capacity to stimulate protective oxygen radical scavengers. Apoptosis induced by cell damage, via intrinsic pathway, implies redox imbalance or even direct oxidative stress (Gao *et al.*, 2005). Therefore, radical-scavenging MEL property is the mechanism correlated to its anti-apoptotic activity, confirmed by the observation that MEL concentrations required for radical scavenging are closely compared to the anti-apoptotic ones (Radogna *et al.*, 2007). In conclusion, this study is a demonstration of MEL protective role from ROS increase induced by H_2O_2, at apoptotic concentration.

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Collenchyma and sclerenchyma in *Ampelopsis brevipedunculata* tendrils

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Summary
Vines like *Ampelopsis brevipedunculata* (vitaceae) produce tendrils which are motile and flex in a circular motion, seeking solid supports. Then, when a support is found they adhere firmly by coiling. To achieve these two functions tendrils use a cellular and developmental mechanism which involves microscopic alteration of cell wall composition and physical properties. Immature tendrils are flexible because their supporting tissues include abundant collenchyma and un-lignified primary cell walls, which allow considerable movement. Mature tendrils acquire large amounts of lignified tissue, become inflexible and decay resistant. This lignified tissue retains cell contents and is composed of fibers not tracheids.

Key words: *Ampelopsis brevipedunculata,* tendril, lignin, collenchyma, fiber.

Introduction

The gross anatomy and physiology of tendrils has been extensively but intermittently studied since the nineteenth century (von Sachs, 1887; Darwin, 1891, 1911; Jaffe and Galston, 1968 and many others). Tendril research continues mainly as molecular (Boss and Thomas, 2000; Hofer *et al.*, 2009) and physiological (Falkenstein *et al.*, 1991) experimentation. However, cellular and histochemical papers are much less abundant in the scientific literature (Lisk, 1924; Meloche *et al.*, 2007; Bowling and Vaughn, 2009)

The most commonly studied system is tendrils of the cucurbitaceae (Dastur and Kapaida, 1931) but these have a significantly different anatomy to tendrils of vitaceae (Bowling and Vaughn, 2009) including the subject of our research; *Ampelopsis brevipedunculata* (Porcelain Berry). This difference takes the form of an uneven distribution of vascular bundles and linear/concave appearance in transverse sections rather than the circular cross section of vitaceae tendrils.

Tendrils (Figure 1A) are a common feature of the vitaceae including *A. brevipedunculata*. This vine is native to northeast Asia and has been introduced to many countries for use as a landscaping plant. After introduction to the United States in the 1870, *A. brevipedunculata* escaped cultivation, and became invasive in many regions of the United States (Young, 2005). It is a weed which climbs at the expense of desirable trees and makes these trees vulnerable to wind damage by increasing wind-drag but making no contribution to stem strength. This causes increased storm damage and occasionally tree fall. A strong and resistant tendril is an essential organ for *A. brevipedunculata's* exploiting life strategy.

Materials and Methods

Common chemical reagents were purchased from VWR International (Bridgeport, New Jersey). Phloroglucinol (JT Baker JTU024-5) was used at 2% $^{w}/_{v}$ in 33% $^{v}/_{v}$ methanol and 33% $^{v}/_{v}$ HCl to stain for lignin, the brittle component of mature secondary cell walls.

For the polychromatic stain Toluidine Blue O (TBO, O'Brian *et al.*, 2004) a solution of the solid (JT Baker JTW143-3) was dissolved in 50 mg/mL in 50 mM citric acid buffer pH 4. Environmental samples of tendrils from live material colonizing vegetation near Whale Pond Brook (West Long

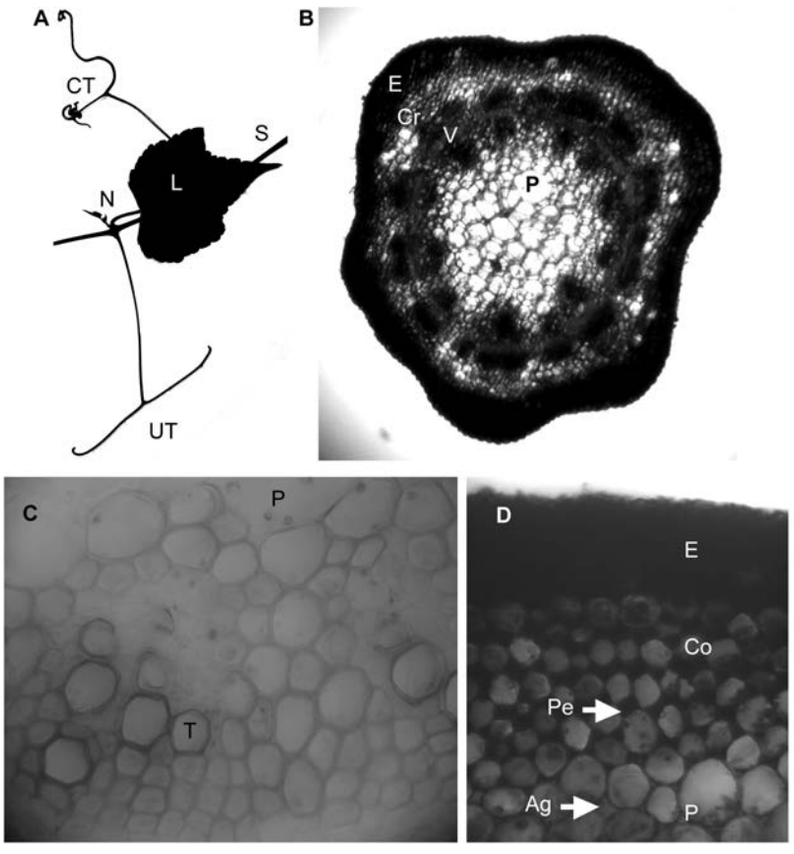

Figure 1. (A) Drawing of immature A. brevipedunculata tendrils. An untouched tendril (UT) reaching for support, coiling tendril (CT), node (N), stem (S) and leaf (L) are shown. Adapted from a photograph of the study material. (B) Immature tendril section showing even distribution of vascular bundles. The multilayered epidermis (E), vascular bundles (V), cortex (Cr) and pith (P) are shown. TBO stained transverse section x100. (C) Thin walled tracheids (T) which stain poorly for lignin. Phloroglucinol stained transverse section x400. (D) Sub epidermal collenchyma (Co) with air gaps filled by pectin (Pe) which appears a roughly rectangular and intensely stained shapes. Parenchyma air gaps are shown (Ag). TBO stained transverse section x400.

Branch, New Jersey) were harvested using a razor blade. These included uncoiled and roughly linear tendrils reaching for support, and tendrils which had coiled on a support but were still green. This live plant material was placed in a styrofoam clamp (Carrington, 2004) and sectioned by hand. The clamp and blade was lubricated with water and sections stored in water before staining. Sections approximately 50 µL were obtained.

For staining with phloroglucinol, sections were placed in a drop of water on a microscope slide and excess water was blotted away with a tissue. A drop of phloroglucinol stain was added then a cover slip applied before the material was observed under bright field using a Nikon YS2-H microscope. For TBO staining sections were placed in a drop of water on a microscope slide and approximately 2 µL stain solution added using an automatic pipette. Dilute stain was mixed using the plastic tip then removed with a tissue. Sections were repeatedly washed with a drop of water and a cover slip applied then the material was observed under bright field as above. Revealing slides were sealed with transparent varnish (Nutra Nail®) and photographed the next day.

Results

Sections of immature untouched tendrils show a multicellular epidermis and a regular arrangement of vascular bundles (Figure 1B). Phloroglucinol staining is very poor and only thin walled tracheids were seen to stain (Figure 1C). Helical thickening of primary cell walls is visible in some sections (data not shown). A conspicuous feature of young tendrils is sub-epidermal collenchyma, which was revealed by TBO staining (Figure 1D). This tissue has stained pectin in place of intercellular air gaps, gluing cells together in a strong but flexible layer.

Mature coiled tendrils which have attached to a solid support (Figure 2A) have a very different histochemistry. They react strongly with phloroglucinol to show thickened layers of cells which superficially resemble stem wood, surrounded by smaller isolated groups of lignified cells (Figure 2B). These cells have thicker walls than immature tendrils (Figure 1C and 2C) but are not water-conducting tracheids because TBO staining shows that almost all of them retain cell contents (Figure 2D). Only a small proportion of

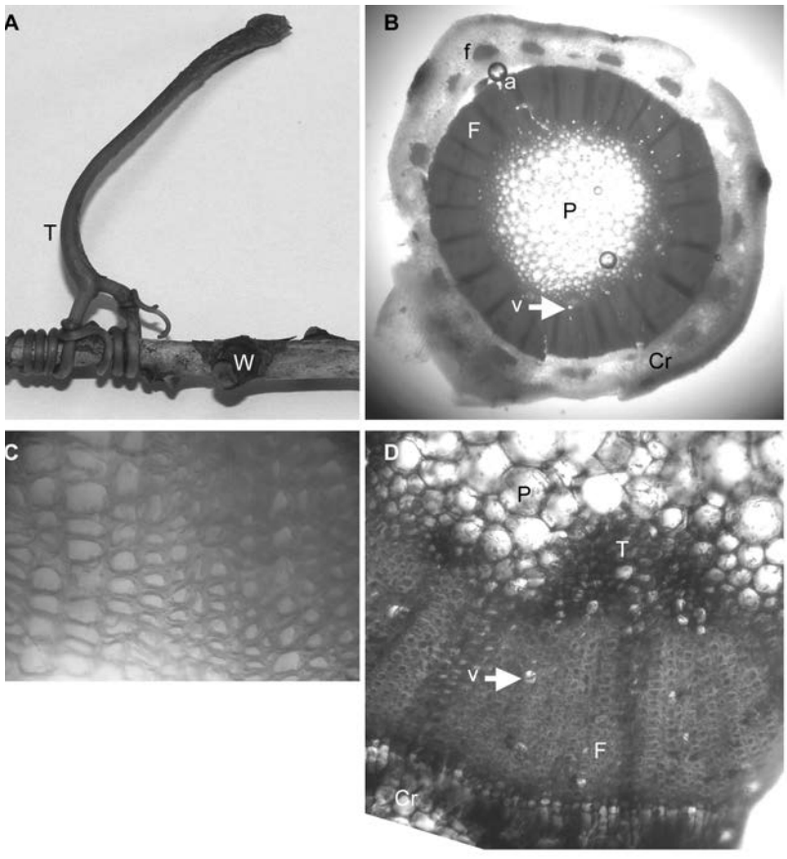

Figure 2. (A) Drawing of one mature A. brevipedunculata tendril (T) coiled around a Salix × pendulina Wenderoth (Weeping Willow) branch (W). Adapted from a photograph of the study material. (B) Mature tendril section showing peripheral bundles of fibers (f) and concentric layers of fibers (F). Occassional water conducting vessels (v) and air bubble (a) artifacts are seen. The cortex (Cr) and pith (P) are shown. Phloroglucinol stained transverse section x100. (C) Phloroglucinol stained fibers with thickened cell walls. Transverse section x 400. (D) BO stained tendril showing fibers with clearly stained cell contents (F). This organ shows occasional vessels (v). Parenchyma pith (P), tracheids (T) and cortex (Cr). Transverse section x 400.

hollow vessel elements are seen (Figure 2B and 2D).

Discussion

Conventional explanations of tendril function include an asymmetric anatomy and differential cell expansion by cells of the cortex and pith, causing curvature around a line or shallow curve of vascular bundles (Jaffe and Galston, 1968). This explanation uses plants of the cucurbitaceae as chief examples (Dastur and Kapaida, 1931). Our results show detailed anatomy of *A. brevipedunculata* tendrils, which do not support this simple view. Tendrils of this species have a symmetrical anatomy in transverse section in both immature and mature states

The supporting tissues in immature *A. brevipedunculata* tendrils are primary xylem and collenchyma which retains flexibility and allows the sweeping movements typical of tendrils (Darwin, 1911). When a support has been grasped, tendrils mature rapidly and the supporting tissue develops large amounts of inflexible fibers. These fibers are easy to confuse with tracheids but fiber cell contents are seen with TBO staining, very different to tracheids and vessel elements which must mature as hollow cells in order to conduct water. Vessel elements are seen, but are a very small proportion of mature tendril cells. Lignified organs have the advantage that they can continue functioning after cell death and tendrils which have lost all tissues except lignified fibrous material, such as epidermis and cortex, still function as adhering organs. Research on the mechanism of *A. brevipedunculata* curvature is continuing.

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