



Chair:  
**Prof. Chiara Castiglioni**

## DOCTORAL PROGRAM IN MATERIALS ENGINEERING

The courses of the Doctoral Program provide Ph.D students in Materials Engineering with a common, basic knowledge on Materials Science and Technology, followed by a specialized training in specific fields. The objective is to combine the theoretical knowledge with the skills required by technology in order to form qualified researchers who can manage the design, manufacturing and use of traditional and/or new materials.

### Contents of the Doctoral Program -

The Doctoral Course covers the following areas:

*Polymers and composites*

*Cements and ceramics*

*Metals*

*Biomaterials and materials for biomedical applications*

*Processing and characterization of advanced metallic alloys*

*Corrosion and durability of materials*

*Innovative materials for civil and industrial engineering*

*Materials Characterization (Microscopies, Scattering, Spectroscopy)*

*Modelling and theoretical approaches to the study of materials structure and properties*

*Micro and nanostructured materials*

*Functional materials for applications in photonic, electronic and sensors*

*Surface engineering and advanced coatings*

*Materials for industrial design*

*Meta-materials*

*Transformation of materials*

*Material for Cultural Heritage*

The courses face immediately advanced issues both in the main and elective courses. Different curricula are offered, which will be activated on the basis of the decisions of the faculty. The Doctoral program is characterised by high flexibility, in order to satisfy the needs of students that have to develop their research activity in different thematic areas. For this reason each student chooses the Introductory and Elective courses and submit to the Faculty a "Curriculum" to be approved. The Courses offered deal with theoretical, experimental and modelling aspects. Several Courses comprise workshops and seminars, with the participation of invited internationally celebrated speakers. Students can use the most modern facilities for materials synthesis, processing and characterization available at the Politecnico di Milano or in other research laboratories.

The research activities related to the Ph. D theses develop, full time, along the three years of the Ph.D. courses. Stages in industries or in national laboratories in Italy or abroad are offered. Each Ph.D. student are supervised by a professor of the faculty: the faculty evaluates the

progress of his research activity at least twice a year and at the end of each year expresses a judgement on the continuation of the work for the subsequent year. At the end of the third year the Ph.D. student presents and defends, in a public hearing, his thesis work with a jury formed by four experts. The Ph.D. student is strongly encouraged to attend at national and international meetings and present papers in his specific field. The Ph.D. student is also assigned to give seminars on topical issues and/or to lecture on the specific fields of his research or on the cultural aspects related to his thesis.

The Doctoral School requires the acquisition of 180 credits (in 3 years) :

- 60 credits must be obtained through attendance at Courses (with positive evaluation in the examinations, when required), attendance at Conferences and International Schools.

- the other 120 credits will be assigned on the basis

of the research activity necessary to the development of the Thesis project.

### Professional skills achieved by PhD in Material Engineering:

The industrial world depends necessarily on a great variety of materials. Nowadays, it is easy to outline two industrial needs: i) development and innovation in the production, processing, application and conservation of traditional materials; ii) development of innovative materials for the production of new manufactured goods or devices to cope with the growing demands of modern technologies. Since these two kinds of industrial needs require specialized people a few specialized curricula are offered. On the other hand, the capabilities acquired through the courses, the research work and the teaching activity assure an adequate preparation to the academic career.

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## MOLECULAR IMAGING OF DISEASE RELATED MISFOLDING OF AMYLOID PROTEINS

Alessandra Apicella

The possibility to image matter in the direct space, down to the atomic level enabled the investigation of biological systems at a molecular level, opening the way of nanobiotechnology and nanomedicine. Among many questions still to be solved, one in particular regards how the environment can influence the interaction between biomaterials and biomolecules, sometimes altering the vital function of the system, as a consequence of a rearrangement induced by the interface. In particular, proteins carry out their function in the human body through a correct folding process, leading to the formation of an energetically stable native state. During this process, proteins may undergo a misfolding leading to aberrant interactions and to the consequent formation of aggregates. This mechanism is correlated to several diseases and understanding the misfolding process is the key to know and defeat the related pathology.

Atomic Force Microscopy (AFM) was here used to investigate the folding process of Ataxin-3 (AT-3), protein responsible of a neurodegenerative disease named Spinocerebellar Ataxia Type 3. AT-3 variants under investigation were the structured domain of the protein alone (AT-3/182Δ, Josephin domain), the

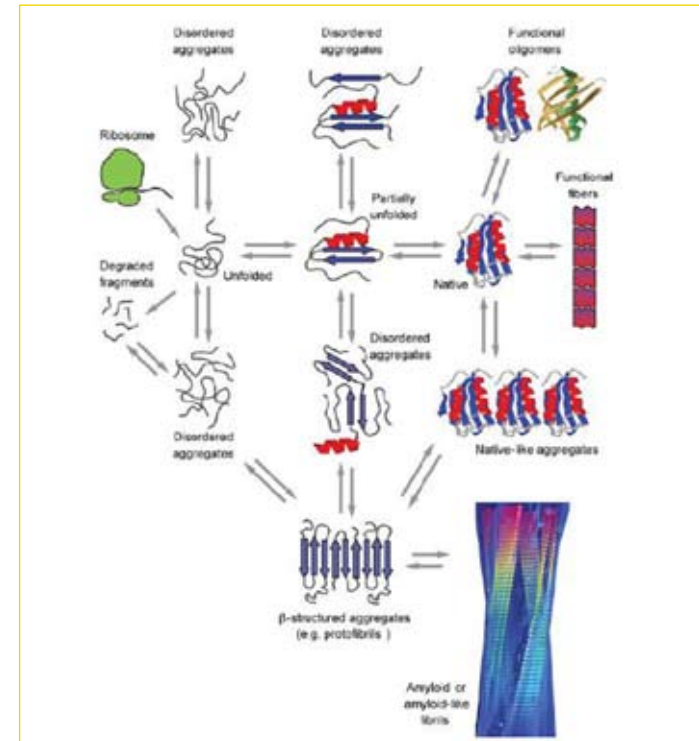
form truncated at 291 residues carrying an unstructured tract, the not expanded physiological form (AT-3Q24), different expanded forms (as AT-3Q40 and AT-3Q55). AFM allowed to obtain a high resolution direct imaging of each folding/misfolding step of AT-3 wild variants and subjected to thermal treatment (85°C), accelerating misfolding, in order to understand the role of each tract of the protein in the process. It was found that all wild variants exhibit globular shape. Instead, increasing temperature the aggregation was mediated by the different tracts of protein chain. Josephin domain mediates the formation of small oligomeric elongated structures, while not expanded poly-Q tract is responsible of the presence of annular aggregates already seen for other disease related proteins. Expanded poly-Q tract is responsible of a complete denaturation when subjected to a strong thermal treatment.

In order to analyze each step preceding the protein denaturation above described, the investigation was then focused on the expanded form AT-3Q55 subjected to long time of incubation in physiological conditions (37°C). The analysis started by a comparison with the behaviour of AT-3/182Δ Josephin domain in the same



1. High resolution dynamic AFM image of AT-3Q55 acquired in air on mica substrate at room temperature. Sample solution was kept at 37°C for 48 hours before deposition.

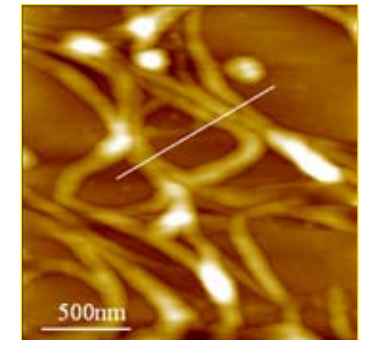
experimental conditions. It was demonstrated that wild AT-3Q55 has globular shape as well as AT-3/182Δ, but although AT-3/182Δ continues to exhibit the same form also after 48 hours of incubation in physiological conditions, AT-3Q55 is characterized by different aggregation steps. In fact, oligomers were observed in the first hours leading to protein plaques and amyloid fibrils formation after 48 hours of incubation (figure 1). Results from these two correlated studies demonstrated that AT-3 is subjected to a two steps folding pathway as proposed by Ellisdon and co authors; in fact, a first step of aggregation, accessible to both not expanded and expanded variants, is mediated



2. A schematic representation of some of the many conformational states that can be adopted by polypeptide chains. The transition from oligomeric aggregates to amyloid fibrils can occur by addition of either monomers or protofibrils (depending on the protein)

by Josephin domain, leading to the formation of small oligomeric structures. Such oligomers were demonstrated to be toxic and they are considered disease precursors. Expanded variants only have access to a second step of aggregation leading to amyloid fibrils formation. Not stable oligomeric structures evolve towards energetically stable amyloid fibrils. Thus, amyloid fibrils formation represents the result of an alternative pathway with respect to native state formation, a consequence of the intermolecular interactions leading to protein misfolding (figure 2). Furthermore, in order to evaluate the role of the

environment in AT-3 misfolding, the Josephin domain behaviour when adsorbed on surfaces with different chemical and physical properties (mica, silicon, gold and gold covered by 1-dodecanethiols) was studied as a model for amyloid protein misfolding. AFM images revealed different forms of aggregation depending on the substrate used for the deposition, opening also the question about the role of the interface in folding/misfolding process. In particular, it was found that the adsorption on hydrophobic substrates contributes to wild AT-3/182Δ misfolding, leading to the formation of protein filaments and bead-like chains (figure 3). On the contrary,



3. Dynamic AFM image of wild AT-3/182Δ on silicon substrate acquired in air.

since the interactions between AT-3/182Δ after different hours in physiological conditions and hydrophobic substrates are not strong enough with respect to the intermolecular interactions between protein monomers, only globular oligomers were imaged.

Results about the effects of thermal treatment on AT-3 variants were sustained by Fourier Transform Infrared Spectroscopy (FT-IR). Furthermore, together with FT-IR Molecular Dynamics (MD) simulations helped in the interpretation of AT-3/182Δ behaviour when adsorbed on substrates. Indeed, the role of unstructured part (182-291 residues) in AT-3 misfolding process was investigated with respect the interaction with substrate through a comparison with the behaviour of Josephin domain.

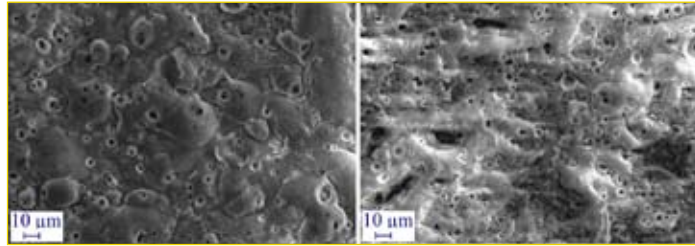
## STUDIES ON SURFACE TREATMENT OF LIGHT METALS

Anna Da Forno

Studies on micro-arc anodization were performed on AM60B magnesium alloy. An environmentally friendly electrolyte containing phosphate, silicate and borate was used for this study with and without addition of nanoparticles of titanium, zirconium and aluminium oxides.

The breakdown voltage for this electrolyte and this substrate is around 100 V, both in absence and in presence of oxides nanoparticles. Voltages in the range 120-140 V are suitable to produce in around ten minutes anodic films whose average thickness is  $\leq 10 \mu\text{m}$ . Regardless to the cell voltage and the oxides thickness, the anodic coatings are porous and amorphous. However scanning electron microscopy (SEM) images of the samples produced in zirconia (Figure 1, left) and alumina containing solutions show that the surfaces have a "fused aspect" due the high temperatures reached within the plasma.

The pores are likely the traces of the sparks and the round shape structures are the result of bubbles expelled from the oxide during fusion, solidified after cooling. On the contrary, SEM images of oxides obtained in nanoparticles free solution (Figure 1, right) and in presence of titania nanoparticles show that oxides are porous, but



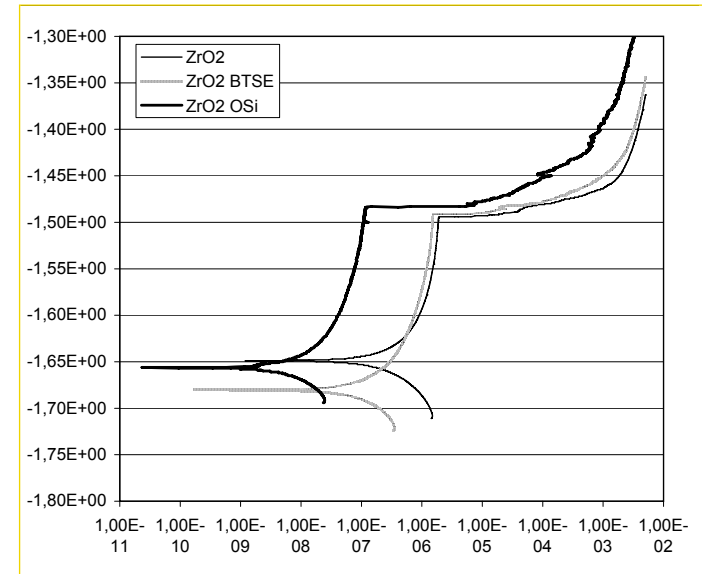
1. SEM surface morphology of a sample produced in zirconia containing electrolyte (left) and in nanoparticles free solution (right).

these round shape structures are less evident. The presence of elements such as silicon and phosphorus, titanium and zirconium detected in the anodic oxides proves the incorporation of elements from the electrolyte during the spark anodizing process.

Experimental results showed the efficacy of the nanoparticles oxides  $\text{ZrO}_2$  and  $\text{Al}_2\text{O}_3$  in increasing both the adhesion to the substrate and the scratch hardness of the micro arc anodic oxides. In fact, while in the oxides produced either in nanoparticles free solution or in titania containing solution gross spallation is detected at very low loads, the behaviour of the coatings produced in  $\text{Al}_2\text{O}_3$  and in  $\text{ZrO}_2$  containing solutions is quite different. At low loads, spallation is not present, however, at applied loads higher than a critical value, a series of cracks running perpendicular to the scratch edges are found in both samples. Their morphology can be associated to tensile

cracking due to the redistribution of stress induced by plastic deformation.

The porosity of the samples has been sealed by means of different surface treatments: sol-gel, organic painting and silane deposition. All the techniques were able to provide further protection to the magnesium alloy, significantly decreasing the corrosion current densities in comparison with the bare alloy and the anodized samples. Sol-gel process and painting were performed onto samples produced in the base solution while silane deposition was carried out onto samples produced in the base solution and in solutions containing  $\text{ZrO}_2$  and  $\text{Al}_2\text{O}_3$  nanoparticles. The sol-gel process was performed by means of two different solutions and the performance concerning the corrosion resistance was similar for both the solutions in relation to corrosion current densities, but the coating produced using the solution at higher pH showed



2. Anodic polarization scans for anodized AM60B substrates in zirconia nanoparticles containing solutions ( $\text{ZrO}_2$ ), anodized and treated with octyltrimethoxysilane OSi ( $\text{ZrO}_2$  OSi) and anodized and treated with 1, 2-bis [triethoxysilyl] ethane BTSE ( $\text{ZrO}_2$  BTSE). Scan rate  $10 \text{ mV min}^{-1}$  in 3.5% NaCl  $25^\circ\text{C}$ .

a passivation zone not present in the coating produced at neutral pH. However, the performed treatment could be not suitable for industrial applications because it takes too many hours for solutions preparation and curing process. Commercial acrylic and polyester painting showed the same performance in the increasing corrosion resistance.

This treatment could be suitable for industrial applications but the heat treatment performed to remove moisture and air from the pores made the panels defective at visual inspection. Silane deposition was performed by using two different silanes as precursors, namely octyltrimethoxysilane (OSi) and 1, 2-bis [triethoxysilyl] ethane (BTSE). SEM cross-section examination of OSi- and BTSE-coated substrates showed that the former covers better

the anodic oxides surface and penetrates deeper the cracks originated in the anodization process. These observations support the trend recorded in the polarization scans (Figure 2).

Long-term immersion tests in 3.5 wt% NaCl were performed at room temperature for a period of 7 days, according to ASTM G31 recommendations. The tests further confirmed the previous considerations. SEM surface examination shows also that worst protection is obtained with BTSE, as indicated by larger and more irregular pores (initially well-defined as produced in the anodization process) than those on OSi-modified surface. Silane treatment was able to provide good appearance coatings in a short process time as well as improved corrosion resistance. Therefore, among the three different surface

treatments, silane-based coatings were preferred as suitable for industrial applications. The anodizing in oxides nanoparticles rich solutions ( $\text{ZrO}_2$  or  $\text{Al}_2\text{O}_3$ ) followed by a silane top coat treatment performed using OSi as precursor is an interesting way to synthesize adherent and corrosion resistant coatings on magnesium alloy AM60B.

An investigation was performed onto anodic alumina produced by means of a two-step anodizing process in order to evaluate the chemical composition and structure of anodic oxides by means of time of flight secondary ions mass spectrometry (ToF-SIMS). The ToF-SIMS data provided some results concerning the morphology of the oxides produced in phosphoric acid solution and in sulphuric acid solution.

# DESIGN OF PHOTOCROMIC DITHIENYLPERFLUOROETHENES FOR OPTICAL MEMORIES

Giovanni Dassa

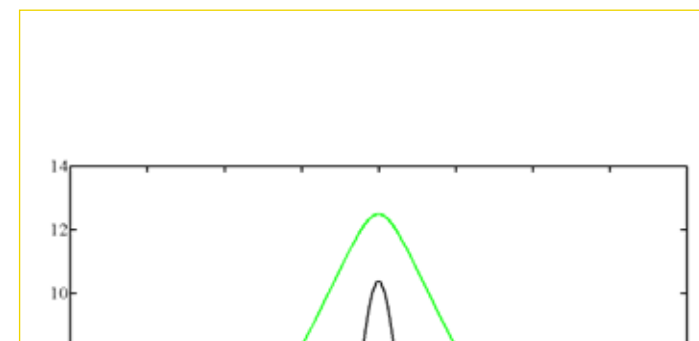
Over the past decades, the research in organic functional materials has received increasing attention for applications in electronics and photonics. Indeed, these materials would provide a lot of potential advantages over traditional technologies, as the environmental-friendly processability and the functioning at the molecular level. In organic smart systems there is a strong connection between electro-optical properties and chemical structure; therefore, the comprehension of the structure-property relationships results to be an essential step to develop innovative devices with optimised performances. Among functional materials, photochromic systems are good candidates for the development of new optical devices based on the reversible light-triggered isomerisation between two states. These isomers are usually characterised by a different extension of  $\pi$ -conjugation which turns into a change in colour and in many other physical-chemical properties. Photochromic 1,2-dithienylethenes have been chosen for developing multistate optical memories since they show important requirements for reliable optical devices, as good thermally irreversible photochromism, high fatigue resistance and

fast response time. In order to develop photochromic multistate memories, at least two molecules showing absorption bands different enough to enable their independent addressing are required. This work deals with the i) optimisation of the chemical structure of a pair of dithienylethenes to address four memory states; ii) production of photochromic materials with good optical properties and iii) development of a non-destructive readout method.

The optimisation of the chemical structure was related to the tuning of the  $\pi$  conjugation of the photochromic molecules in both the isomeric forms, by the addition of aryl groups to the basic dithienylethene structure and/or the use of electron-active substituents. The effect of the variation of the chemical structure was studied by UV-visible spectroscopy. It was found that inserting phenyl or thienyl rings at the 5,5' position of the basic 1,2-bis-(2-methyl-3-thienyl) perfluorocyclopentene results in a strong bathochromic shift of the actinic band in the visible. The use of electron-active groups provided some effects: using donor groups a red-shift occurred, whereas using withdrawing groups the situation was more complex. Indeed,

if the group had  $\pi$  electrons that enable extension of conjugation, a red-shift took place. The presence of a donor-acceptor structure provided a larger red-shift thanks to a partial intramolecular charge transfer character. It was worth noting that the shift is dominated by the extension of conjugation while the electron-active substituents play a less relevant role. Finally, two series of photochromic molecules were obtained, namely a group with a maximum of the visible absorption band at about 515 nm and a group with a peak at about 590 nm. One molecule for each series was selected to develop a bicomponent system.

Aimed at developing an optical device, photochromic materials with suitable optical properties, namely glassy, easily processable and showing high output signal, were required. Two possible methods have been proposed. The simplest route was the dispersion in a glassy matrix: accordingly, chromophores were dispersed in a PMMA or in a sol-gel precursor matrix. In the latter case two different kinds of matrices were analysed, basic and acid. The basic matrix promotes some degradation phenomena of the photochromic species, while good results have been obtained with the acid one. The second approach was



1. Pattern written in the visible (a) and pattern read in Mid-IR (b)



2. Addressing scheme for the photochromic polyurethane containing two active unit

the synthesis of polymers containing the chromophore in the main chain. Photochromic polyurethanes were obtained using hydroxyl end-capped dithienylethenes and polyurethanes with one or two photochromic species have been produced. Their optical properties were good as well as the photochromic response.

The readout methods developed in this work exploited the change in vibrational features of 1,2-dithienylethenes, which occurs during the photo-induced process.

As for readout based on IR signal, two asymmetric dithienylethenes with different UV-visible absorption spectra were dispersed in PMMA. It was found that four states

were addressable in this system; moreover, the changes in the IR spectra were large in a wide spectral region, making possible to read the stored information by using an IR camera that measures the integrated intensity in a wide range of wavenumbers. This provides an advantage for technological applications. By transferring a pattern on the active substrate by converting the material among the different forms it was possible to detect three different states (Figure 1).

The readout method based on Raman spectroscopy was tested on polyurethane films containing two photochromic monomer units. The optimisation of the ratio between the two chromophores turned out to be a key factor to detect the four addressable states (Figure 2).

Finally, it was possible to propose a setup for a memory device with non-destructive Raman scattering readout by using a laser at 1064 nm, two multilayer filters to remove the scattering Rayleigh and to separate the contributions of the two photochromic units, and two photodiodes.

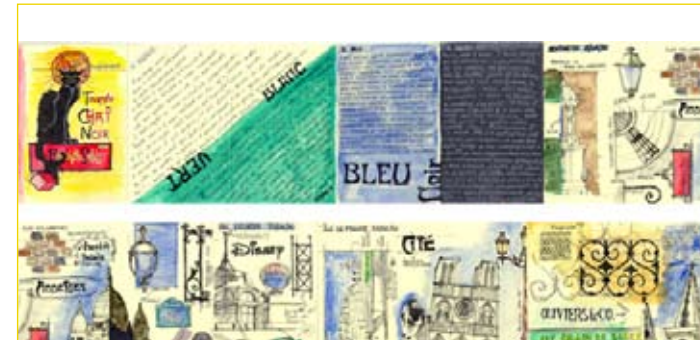
## ULTRAFINE GRAINED ALUMINIUM ALLOY PRODUCED BY SEVERE PLASTIC DEFORMATION

Stefano Farè

Aluminium alloys are potential candidate to substitute carbon steel in many structural application. The limiting factor, so far, has been represented by the lower mechanical properties and formability. Last property is strictly influenced by the texture emerging after plastic deformation or heat treatments. In particular, for rolled flat products, carbon steel exhibits a  $\gamma$ -fiber texture, which has high value of Lankford parameter, allowing a good deep drawability. Aluminium and its alloys exhibit a  $\beta$ -fiber texture, which has a low value of planar anisotropy. Since 50s and thanks to Hall and Petch's work, it is well known that mechanical properties can be strongly enhanced by refining the microstructure. Since aluminium and its alloys don't show phase transitions, the only possible mechanism to exploit to refine the grain structure is the plastic deformation. Deformation at high level of strain (commonly superior to 400%) is usually defined as severe, and it allows to activate the continuous dynamic recrystallization (cDRX), which enables the formation of an ultrafine grain structure only at room or moderate temperature. This phenomenon allows to fragment grains at high strain, thanks to a continuous rotation

of subgrains and dislocation cells, which can be formed in a high stacking fault energy metal as aluminium and its alloys. The terms continuous refers to the fact that nucleation and growth are undistinguishable. Ultrafine grain size can also promote superplasticity. Superplasticity is the capability of some metals and alloys to undergo extensive plastic deformation without necking. It is well known that the strain rate in superplastic regime is related to the grain size by a factor of  $1/d^2$  which means that, by reducing the grain size of one order of magnitude, the strain rate increases of two. Among numerous techniques developed to achieve severe plastic deformation regime, only rolling might be exploited industrially. In particular, asymmetric rolling (ASR) and accumulative roll bonding (ARB) represent the most interesting and promising. Asymmetric rolling is a technique in which the circumferential speed of the two rolls is different (figure 1), thus letting the material experience an asymmetric state of strain, with a strong shear component. ARB is a discontinuous process that keeps the geometry and size of the billets constant. In ARB, the material is subjected to a specific procedure, which can be repeated. Firstly, the material

is cut and the surfaces to be joined are roughened and cleaned with solvents in order to remove grease and particles. The two parts are then stacked together, sometimes slightly heated and are rolled again. In this work, the possibility of strongly refining the microstructure of a 6082 type aluminium alloy has been investigated. The first part of activity has regarded the set up of two rolling processes: ASR and ARB. This topic highlights the most important parameters for each process: asymmetry ratio for ASR (the ratio between the rotational speed of upper and lower rolls) and joining technique for ARB. Both processes have been conducted up to the severe plastic deformation regime. Billets have been rolled starting from a fully annealed condition to better understand the evolution of microstructure as a function of equivalent strain. On the other hand, asymmetrically rolled billets from a solution treated state has allowed to characterize the aging behavior of the alloy subjected to severe plastic deformation, as compared to conventionally rolled samples. Aging maps have been generated. Differences in peak-hardness time highlighted for two temperatures (130 and 180 °C) and different value of reduction (0%, 77% and 98%).



1. Scheme of different strategies to apply asymmetry

Differential scanning calorimetry technique has been used. DSC allowed to identify the temperature at which thermally activated phenomena (as, for example, precipitation and solution of second phase particles) occur. Figure 1 Scheme of different strategies to apply asymmetry. All the rolled billets have been characterized by optical and electron microscopy (both scanning and transmission electron microscopy) to reveal different features and scale of microstructure and its evolution. TEM analyses have permitted to assess the dislocation density, SEM analyses with back scattered electrons channeling have allowed to reveal a qualitative map of crystal orientations, while optical microscopy revealed the grain structure over larger areas.

Figure 2 TEM micrographs of the material rolled with a reduction of 97,66%. The influence of rolling parameters is highlighted. A mechanical characterization has also been performed. Microhardness measurements have been conducted on samples taken during the rolling process and it allowed to correlate the evolution of the mechanical performance and process setups (in terms, for example, of asymmetry ratio for ASR). Measurements have been compared among conventional and non conventional rolling techniques as a function of equivalent strain. As mentioned at the beginning of this abstract, rolling texture of aluminium alloys is a limiting factor. Asymmetrically rolled material has been characterized by XRD to characterize the deformation texture. It was



2. TEM micrographs of the material rolled with a reduction of 97,66%. The influence of rolling parameters is highlighted

demonstrated that a strong shear strain can modify the conventional rolling texture to a more favorable shear texture.

# CROSS-LINKED POLYETHYLENE FOR ORTHOPAEDIC APPLICATIONS: ANALYSIS OF PHYSICAL AND DOPING TREATMENTS FOR ENHANCING THE PERFORMANCE

Daniela Ferroni

Ultra-High Molecular Weight Polyethylene (UHMWPE) has been the material of choice for hip and knee joint replacements over the last four decades thanks to its properties of low friction and good biological compatibility. In order to increase the serviceability and wear endurance of UHMWPE implants, starting from the late 1990s, Highly Cross-linked PolyEthylenes (HXLPEs) obtained by submitting the raw polymer to high doses of irradiation (50 kGy and more) were proposed. The cross-links among molecular chains promoted by irradiation generate a three-dimensional network within the polymer that inhibits chain slippage and makes the UHMWPE more resistant to wear. However, an important issue related to the use of HXLPEs concerns long-term oxidation due to unbonded free radicals produced during irradiation, and the related degradation of the end-user properties. Special procedures have been introduced to overcome this problem, based on two alternative approaches: (a) stabilization of irradiated HXLPE by thermal treatments which promote recombination of free radicals, or (b) doping of raw PE powder with antioxidant agents like  $\alpha$ -tocopherol (vitamin E) before compounding and irradiation. Today HXLPEs stabilized with

thermal treatments are already in clinical use, while  $\alpha$ -tocopherol-doped PolyEthylenes are still at the stage of pre-clinical evaluation; however at present a general consensus about the optimal procedure does not yet exist among orthopaedic manufacturers and clinicians. Within this framework, the Thesis work is focused on the investigation of the morphological changes introduced in the structure of UHMWPEs by the different procedures and the related changes in mechanical properties. The final aim is to provide general recommendations for the optimal combination of cross-linking and stabilization procedures in order to achieve the target functional properties of HXLPEs. The present work is expected to represent an original contribution in the assessment of HXLPEs for the community of Materials scientists. Most of the engineering studies carried out in the recent years on HXLPE were performed by investigators in biomechanics and focused exclusively on the analysis of the mechanical performance of the final product, while the assessment of the morphology changes produced by the cross-linking procedure was not a primary focus. Another limit of

current studies was that they did not investigate the individual effects of either the irradiation and the stabilization procedure, but only the outcomes of their combination.

The raw material considered in the present study is GUR 1020 medical grade UHMWPE (manufacturer Ticona GmbH, Germany) processed by compression moulding by Orthoplastics (Lancashire, UK). The raw material was divided into three samples described in Table 1. The first one was gamma irradiated in air at room temperature at several doses (75, 100, 150, 200, 250 and 300 kGy). The second sample was gamma irradiated in air at 75 kGy and divided in two sub-samples, heated at either 110°C (annealing procedure) or 150°C (remelting procedure). Both thermal treatments were carried out for 2 hours in inert gas atmosphere, followed by slow cooling down at a rate of 1°C/min to room temperature. The third sample was blended with 0.1 wt%  $\alpha$ -tocopherol before compounding. Doped UHMWPE was further divided into two sub-samples: the first was gamma irradiated at 75 kGy, while the second one was not irradiated and tested as such.

ID	IRRADIATION DOSE	THERMAL TREATMENT	VITAMIN E DOPING
UHMWPE	/	/	/
HXLPE	75 kGy	/	/
	100 kGy		
	150 kGy		
	200 kGy		
	250 kGy		
300 kGy			
1 <sup>ST</sup> GENERATION HXLPE	75 kGy	110° C, 2h	/
		150° C, 2h	
2 <sup>ND</sup> GENERATION HXLPE	0 kGy	/	0.1 wt%
	75 kGy	/	0.1 wt%

Table 1. Tested materials

The effect of the individual processes on the morphological properties were investigated according to relevant standard methods. The degree of cross-linking of each sample was quantified by determination of the insoluble polymer fraction (gel content), soluble fraction (extract percentage) and mass swell ratio using a method based on ASTM D 2765. Differential scanning calorimetry (DSC) tests according to ASTM E 794 were carried out to assess the crystallinity and the melting transition temperature of the samples. The UHMWPE oxidation was quantified measuring the density of carbonyl groups in selected 200  $\mu$ m thick specimens according to ASTM F 2102. The mechanical properties were evaluated by uniaxial tensile test, small punch test, Charpy impact test, DMA and wear test. Uniaxial tensile tests were performed according to ISO 527 at room temperature (23 $\pm$ 2°C) at a displacement rate of 50 mm/min. The small punch tests were carried out on disk specimens (0.5 mm in thickness and 6.4 mm in diameter) according to ASTM

F 2183. The Charpy impact tests were performed according to ISO 11542 on notched specimens (two notches with 14 $\pm$ 2° angle) of 120 $\times$ 15 $\times$ 10 mm. The dynamic tests were carried out in three point bending configuration applying a sinusoidal displacement with a constant amplitude of 10  $\mu$ m at 1 Hz while the external temperature was changed from 23°C to 150°C at 1°C/min. Wear endurance was measured through a pin-on-flat test carried out on pins with 8 mm diameter and 25 mm height, rubbed against highly polished Co-Cr-Mo plates according to an "8-shaped" path of 11 cm length. The pins were kept moist in a solution of water (225 mL) and bovine serum (75 mL). The duration of the test was 500,000 cycles. Physical and mechanical tests were carried out both on unaged and aged specimens, the latter prepared by accelerated ageing in full oxygen atmosphere at 5 atm pressure and 70°C for 14 days, according to ASTM F 2003.

The main outcomes of the work can be summarized in the

following points:

1. The irradiation dose impacts significantly on the degree of cross-linking, but saturation is achieved at doses on the order of 100 kGy; higher irradiation doses have not practical effect on the cross-linking density but promote severe oxidation;
2. The thermal stabilization performed at a temperature below the melting temperature of UHMWPE increases the crystallinity content but is not able to completely preserve the final product from long term oxidation;
3. The thermal stabilization performed at a temperature above the melting temperature of UHMWPE removes free radicals and increases oxidative resistance of HXLPEs;
4. Blending the raw UHMWPE with a small amount of vitamin E (0.1% in weight) is effective in preserving the HXLPE from long term oxidation;
5. The increase in cross-linking density is related to increase in wear endurance but at the same time reduces the toughness and ultimate mechanical properties of first generation HXLPEs;
6. The increase in crystallinity is related to increase of elastic modulus and yield stress;
7. Presence of oxidation products is associated to embrittlement of the product in long term;
8. Vitamin E doping has not adverse effects on the increase in wear endurance promoted by cross-linking, but preserves the final product by the decrease in toughness exhibited by first generation of HXLPEs.

# SCRATCH BEHAVIOUR OF POLYMERS IN RELATION TO THEIR VISCOELASTOPLASTICITY

Pinar Kurkcu

Polymers are more and more frequently being used in applications demanding a high surface quality such as in the automotive, data storage and optical industries. "Surface quality" is a complex issue, comprising several features whose relative importance depends on the specific application. From the mechanical point of view one of the important characteristics is the resistance to scratching. Scratches may severely compromise the overall performance of polymer components; beside being aesthetically undesirable, they can also impair the component functionality or even its structural integrity. To enhance polymer durability, it is therefore of crucial importance to understand their scratch behaviour. This study aims to investigate the relationship between the intrinsic viscoelastic properties of polymeric materials and their scratch behaviour. In the first part of the study, the scratch performance of a range of neat polymers, which are expected to exhibit different viscoelastic and scratch characteristics, was assessed. In the second part, the study is extended to investigate the effect of incorporating different amounts of hard and soft fillers on the scratch behaviour of polymeric materials. Scratch tests were performed on a CSM microscratch tester by sliding the indenter at constant penetration depth for various

sliding tip velocities.

The so-called "scratch hardness", which is a mean of the contact pressure exerted by the indenter on the material, is the quantity commonly used to evaluate the scratch behaviour of the materials. The first approach to determine scratch hardness assumes that only the front face of the indenter is in contact with the material. This assumption is valid for purely plastic or elastoplastic materials but not accurate when the viscoelastic-viscoplastic nature of polymers is taken into account. This study focuses on a recent scratch model which takes as a basis a "rheological factor" which compounds the influence of the elastic modulus and the yield stress of the material in determining the true contact area between indenter and material. The model can be enhanced by considering the strain rate dependency of these characteristics. For this purpose, the materials investigated were further characterized by dynamic mechanical test and compression test in order to determine the sensitivity of storage modulus and yield stress to strain rate. By use of the "rheological factor", the elastic recovery angle at the rear side of the contact was estimated and hence the true contact area and the scratch hardness were derived. The adequacy of the model for predicting the elastic recovery and pile-up formation

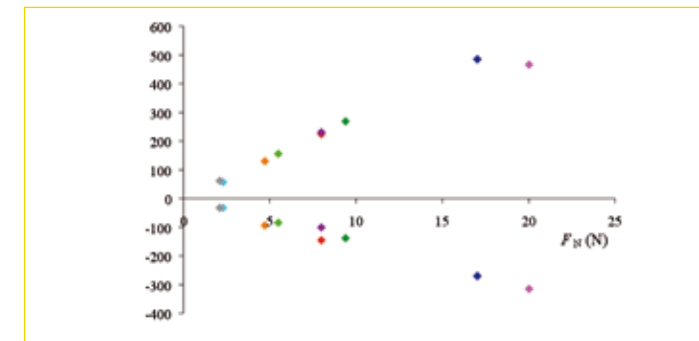
characteristics was assessed by comparing the outputs of the model with the experimental results obtained by analyzing the residual profiles of the scratch tracks.

The scratch phenomenon can be affected by combinations of a variety of material properties and testing conditions and it is difficult to identify the effect of each parameter on the scratch behaviour. The application of the current scratch model on neat materials revealed that scratch behaviour is dominated by the yield stress of the material. However, in particle-filled polymers, yield stress cannot adequately account for the observed scratch behaviour due to the important role of local micromechanical phenomena. Whether the local phenomena or the yield behaviour of the system is dominant is likely to be dependent on the size of the filler. Incorporation of hard fillers results in a significant variation in the deformation mode of polymeric materials. When neat polymers are scratched, the material deforms viscoelastically and viscoplastically and forms "pile-ups" both at the sides of the groove and in front of the indenter. No crack formation is observed. The composites containing glass spheres show a brittle failure with chip and debris formation.

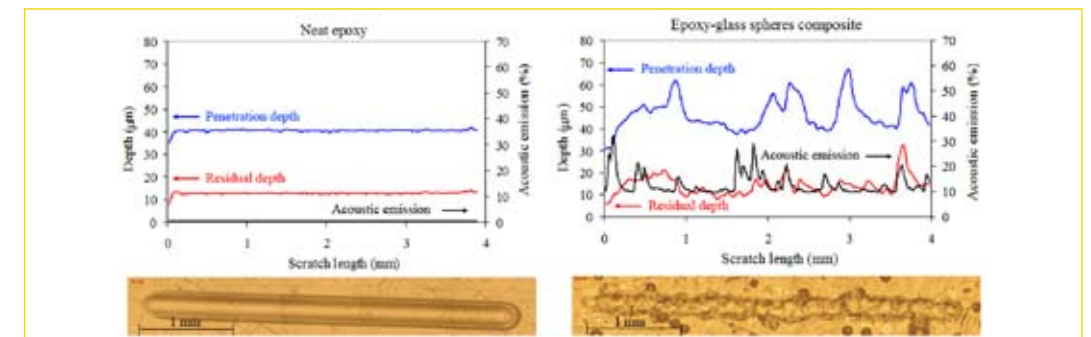
The peaks in acoustic emission records seem to reveal the breakage of spheres whereas the peaks in penetration and residual depth records can be related to the voids that are formed when the spheres are displaced. Addition of hard fillers brings about a slight enhancement in the scratch hardness owing to the increase in the modulus of the material. However, change of deformation mode from ductile ploughing to brittle failure causes

an increase in the scratch visibility. On the contrary, incorporation of soft particles do not seem to have a significant effect on the scratch deformation mechanism of the neat polymers. Soft particles causes a reduction in the scratch hardness as a consequence of the decrease in yield stress of the material. Despite of its crucial importance, the scratch phenomenon is still not well understood. The existing models are not

sufficient to describe the effect of viscoelastic-viscoplastic nature of polymers on their scratch behaviour. Besides, there are few studies on scratch performance of composites, in which local phenomena are significant. This study aims at introducing a primary analysis for evaluating the scratch process in the presence of fillers, which needs to be further investigated in detail.



1. Correlation between scratch hardness and yield stress

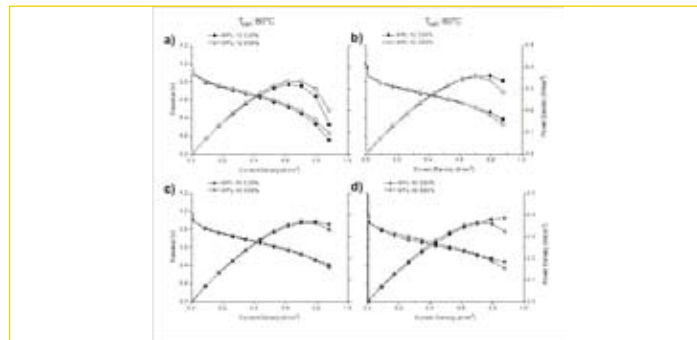


2. Penetration depth, residual depth and acoustic emission records of neat epoxy and composite having 40% vol. of glass spheres ( $d = 100 \mu\text{m}$ )

# PREPARATION, CHARACTERIZATION AND TESTING OF GAS DIFFUSION LAYERS FOR PEM FUEL CELLS

Luca Omati

The research is focused on the characterization and development of new materials for polymer electrolyte membrane fuel cells (PEMFC). The attention has been mainly focused on surface treatments of gas diffusion layers, but not exclusively. Gas diffusion layer (GDL) is a substrate made by carbon clothes or carbon paper, placed between the catalyst layer and the current collector. It ensures a uniform gas distribution over the entire active area and guarantees the electric contact. GDL is usually treated with hydrophobic agents in order to ensure the water repellency of the substrate, necessary to prevent the cell from water flooding. A Micro-Porous Layer (MPL) is usually coated onto one side of GDL (Gas Diffusion Medium, GDM) in order to improve the electric contact with the catalyst layer and manage the excess of water produced by the reduction reaction at the cathode side. The aim of the thesis was to prepare, characterize and test different GDLs coated in different way. The sample analyzed were characterized ex-situ using microscope analyses (SEM or optical), contact angle and electrical resistivity (in plane and through plane) measurements. Finally the GDLs were tested in-situ, i.e. while the fuel cell is running, and

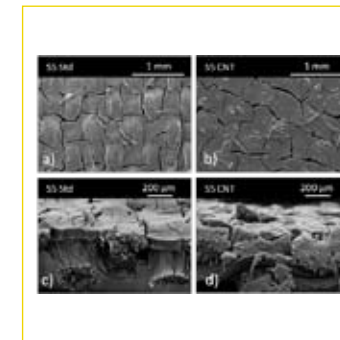


1. Polarization and power density curves of the cell assembled with MPL12, MPL40 GDMs at two levels of compression (i.e. 30 and 50%). The cell operates at 60 °C (a, c) and at 80 °C (b, d).

characterized by polarization/ power density curves and electrochemical impedance spectroscopy measurement. Electrochemical impedance spectrum were fitted with equivalent circuits in order to obtain electrical parameters that represent different fuel cell losses; i.e. ohmic resistance (ohmic losses), polarization activation resistance (kinetic losses) and diffusion resistance (flooding phenomena). In the first part of the thesis different composition of MPLs and different GDLs textile were analyzed. Some experiments were conducted to better understand the effect of MPL and the effect of operative conditions as feeding gas relative humidity, GDL compression and cell temperature. A set of four Gas Diffusion Layers (GDLs) having different textile properties (i.e.

density, warp, weft and weight), produced by an Italian Company (SAATI), and coated with the same Micro Porous Layer (MPL), was investigated with the aim of assessing the influence on the FC performance. The FC ran under different operating conditions (i.e. humidity and gases flow rate) at constant temperature (60°C): three RHs were selected at the cathode side while keeping constant the RH at the anode side. Moreover, all experiments were carried out at constant gas flow rate. The experiments show that the four substrates have quite similar performances and are equally affected by the gas relative humidity. Performing the EIS analysis made possible understand which parameters mostly affect the fuel cell performances. From the textile tested in this part it is possible to observe that thinnest and lightest

GDLs perform well in a vast range of operative conditions thanks to their low bulk resistivity and good compressibility, but they have a reduced capacity of water management. Secondly, different MPL compositions were investigated. In particular, it was analysed the

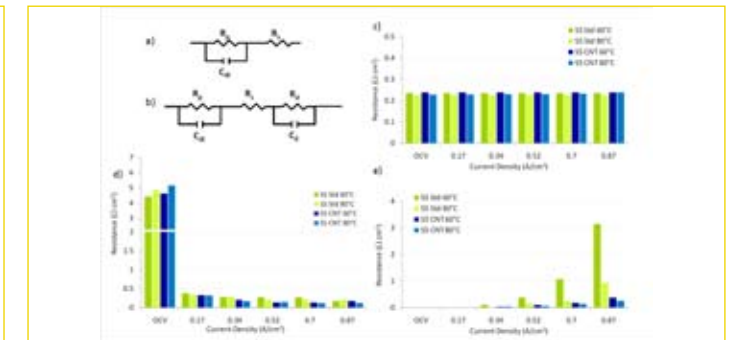


2. SEM images of the surface of the different GDMs S5 Std (standard) and S5 CNT (with partial subs. of CNTs): a, b) surface morphology, c, d) sample thicknesses

effect of different amounts of hydrophobic agent i.e. 12 and 40% PTFE content into MPL composition. Not only MPL composition, but also its compression affects FC performances; so, analysing different GDLs compression ratios, it is possible to optimize the fuel cell assembly. Polarization and EIS analysis were carried out to understand the effect of the presence of MPL onto the performances of the fuel cell. the different samples were analyzed at different compression ratio i.e. 30% and 50% of the initial thickness.

Polarization curves show that high compression ratio has a beneficial effect onto fuel cell performances however the cell is more affected by flooding

phenomena present at high current densities (Fig. 1). Higher PTFE content into MPL helps the cell to expel the excess of water and prevent the flooding effect. Finally the addition of carbon nano-tubes in partial substitution of carbon matrix into the MPL ink formulation was investigated.



3. Electrical circuits used for fitting spectrum data: low current density ( $i < 0.4$  A/cm<sup>2</sup>) a) and high current density ( $i > 0.4$  A/cm<sup>2</sup>) b).and electrical parameter obtained from impedance spectra fitting: ohmic resistance c), Polarization activation resistance d) and diffusion resistance e)

Morphological analysis were carried out to evaluate some differences in Micro-Porous Layer coating. SEM images were performed for the GDM samples in order to highlight some differences due to the presence of CNTs (Fig 2).

The hydrophobicity of the MPL, which had a good influence on FC performances, turned out to stem from a decrease in impedance parameter, especially the diffusion resistance at high current density generated by the cell.

The partial substitution of carbon nano-tubes (CNTs) instead of carbon powder into the MPL had a beneficial effect on fuel cell performances. The sample with CNTs analysed in detail with EIS (Fig 3) showed good results

in terms of resistivity decrease and good water management at high current density. In the last part of the thesis the attention was focused on GDL treatments; a non-conventional coating of perfluoropolyether (PFPE) was compared with the commonly

used polytetrafluoroethylene (PTFE) coating. The results obtained showed that the GDL coatings with PFPEs (polyurethane and phosphate) have a good hydrophobicity as pointed out by the contact angle measurements. The two samples of GDLs coated with PFPEs were assembled in a running fuel cell and compared with a GDL coated with PTFE as reference. When the cell operates at 60°C, the assembly with the PFPE-treated GDL sensibly improved the cell performances. This effect was even more evident in the high current density region, where the cell is more stressed by the water generated by the cathode reaction. EIS measurements confirm that PFPE samples has higher conductivity and good properties of water expulsion.



# DEVELOPMENT AND CHARACTERIZATION OF NOVEL STIMULI-RESPONSIVE MATERIALS FOR GENE DELIVERY

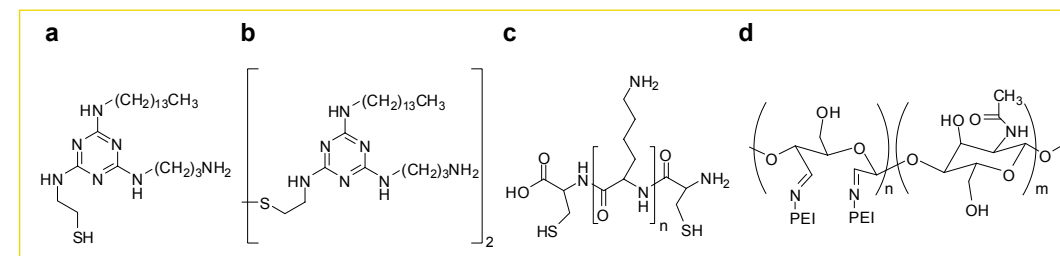
Daniele Pezzoli

Gene therapy can be broadly defined as the introduction of genetic material into cells to obtain a therapeutic effect by altering the cellular phenotype. Offering new treatment possibilities for both inherited and acquired human diseases, gene therapy has become one of the most intensively developing strategies for current clinical research. Since DNA is large and negatively charged, it is not internalized efficiently by cells. Hence, the goal of gene therapy is largely dependent on the development of efficient agents, named gene delivery vectors, able to deliver nucleic acids within cells. Initial research focused on using viral carriers, which exhibited high efficiency of delivering exogenous genetic material to cells. However, safety issues associated with viral gene vectors have increasingly encouraged the design and the study of different potential non-viral gene delivery systems for gene therapy applications. Non-viral gene delivery is a simpler, cheaper and, most importantly, safer alternative to viral vectors. Non-viral vectors are materials capable of spontaneous DNA complexation, providing protection from nucleic acid degradation by nucleases, promoting cell internalization and enabling gene transcription

in the cell (transfection). Over the past twenty years, many advances have been made in the development of non-viral vectors. However, non-viral gene delivery has not yet achieved the gene transfer efficiency of viral carriers. In this project both lipidic and polymeric materials have been developed and characterized to obtain efficient gene delivery systems featuring high physiological tolerance. Spectrofluorimetric analysis were used to investigate the ability of the vectors to complex DNA; Dynamic Light Scattering and Laser Doppler Velocimetry allowed to assess the dimensions and the surface charge (Z-potential) of the vector/DNA complexes. Moreover, the complex stability was evaluated in presence of reducing agents such as glutathione (GSH), mimicking the intracellular milieu, and of biological fluids such as fetal bovine serum, mimicking the extracellular environment. The biological activity of lipidic gene delivery vectors was evaluated *in vitro* in terms of cytotoxicity and transfection efficiency on different cell lines. Finally, structure-activity relationship studies paved the way toward the development of more effective materials.

A triazine-based cationic lipid, SH14 (Figure 1a), featuring a

redox sensitive thiol moiety, was characterized and developed *in vitro* as gene delivery system. Moreover, the dimeric counterpart of SH14, the *gemini* lipid SS14 (Figure 1b), was formulated with helper lipids to obtain reducible 100 nm-liposomes. Both SH14 and reducible liposomes could efficiently complex DNA to form lipoplexes with low dimensions (< 300 nm) and positive surface charge. These lipoplexes were highly stable in serum-enriched cell culture medium, but were disassembled by reducing environment. The transfection efficiency of our lipidic gene delivery systems was similar or higher than that of benchmark lipidic transfectants, even in presence of serum, while cytotoxicity was moderately low. These promising results underlined the importance of the redox-sensitive disulfide moiety in designing highly effective reducible vectors ("disulfide linker strategy"). Of note, among intracellular antioxidants we demonstrated the key role of glutathione in modulating transfection effectiveness through the intracellular disassembly of reducible cationic lipids/DNA complexes. A family of redox sensitive polymerizable dithio-peptides, based on a central L-Lysine (Lys) backbone and two terminal



1. Chemical structure of (a) the triazine-based cationic lipid SH14; (b) *gemini* lipid SS14; (c) the poly-L-lysine-based dithio-peptides Cys-Lys<sub>n</sub>-Cys, n = 6, 10, 14; (d) chitosan-graft-bPEI

L-Cysteine (Cys) residues (Cys-Lys<sub>n</sub>-Cys, Figure 1c) was developed. We demonstrated that Cys-Lys<sub>n</sub>-Cys could polymerize in presence of DNA by template-driven oxidative polymerization, forming highly stable complexes. Newly formed disulfide bonds, as predicted by the "disulfide linker strategy", were cleaved by intracellular reducing milieu favouring polyplex unpacking. The influence of the poly-L-Lysine chain length on both the physicochemical properties and on the biological activity of these vectors was investigated. Cys-Lys<sub>n</sub>-Cys peptides exhibited very low cytotoxicity. Moreover, a minimum consensus motif of Lys<sub>10</sub> was needed for effective delivery of nucleic acids. Finally, aiming to combine the high transfection efficiency of the polymeric gene delivery vector branched polyethylenimine (bPEI) with the biocompatibility and low cytotoxicity of chitosan, low molecular weight bPEI was

grafted to the chitosan backbone (Figure 1d). Although the molecular weight of the copolymer was increased with respect to starting bPEI, the cytotoxicity was limited by the intracellular enzymatic degradation of the chitosan backbone. The effect of bPEI grafting degree on the physicochemical properties of copolymer/DNA complexes and on their *in vitro* biological activity was investigated. Of note, both grafting degree and the concentration of copolymer used to complex the DNA deeply influenced the effectiveness of these vectors. Among the copolymer series, chitosan-graft-bPEI with a grafting degree of 3.3% allowed for increased transfection efficiency compared to bPEI.

In summary, four different gene delivery systems were studied. Vectors were optimized to find the best transfection conditions in presence of serum and the

relationship among chemical structure of gene delivery vectors, the physicochemical properties of vector/DNA complexes and their biological activity were established. We demonstrated that both "disulfide linker strategy" and enzyme-mediated intracellular chitosan degradation were useful approaches to design stimuli-responsive systems suitable for gene delivery applications. Overall, the understanding of transfection mechanisms at the molecular and supramolecular level will underlie the design and the development of more effective materials.

# ATOMIC FORCE MICROSCOPY TECHNIQUES FOR THE NANOLITHOGRAPHY AND SURFACE CHARACTERIZATION OF THIN COATINGS

Luca Torlaj

Nanolithography is a promising technology able to generate patterns with very high accuracy and reproducibility even on nanometric level; it can be performed by means of atomic force microscope (AFM). This technique uses the microscope tetrahedral tip to deliver several and different kind of information (which could be electrical, magnetical, mechanical and chemical) to the substrate surface. The main topic of my PhD project concerned two different nanolithographic techniques: mechanical nanolithography for measuring thin film (with the thickness of few  $\mu\text{m}$ ) nanoscratch hardness, and chemical nanolithography for the realization of genomic micro and nanoarrays.

## Dip-Pen Nanolithography

It has been possible to prepare DNA arrays onto both inorganic surfaces (such as Au, Si Oxide) and organic substrates, specifically thermoplastic polymers such as polystyrene, polycarbonate and polymethylmethacrylate. The latter materials have been studied as new substrate materials for oligonucleotides arrays in order to overcome some problems (reproducibility and surfaces contamination) related to the use of Au surfaces, and to reduce production costs thus opening the way to future Lab-On-Chip development. Oligonucleotides

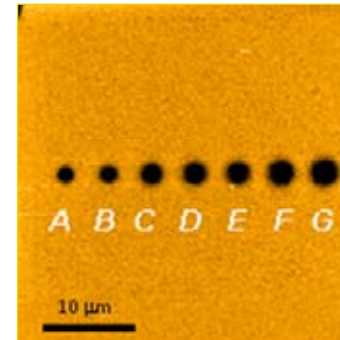
properly functionalized with different end groups (such as acrilamide and amine) have been directly deposited onto spin-coated flat polymer surfaces. It was found that the driving force allowing the deposition and immobilization of the oligonucleotides on the polymeric substrate is a specific interaction between the chemical group bonded to the probe, and the polymeric substrate itself. The study of the diffusion coefficient during the DPN experiment was carried out through a screening of several different ink-substrate pairs with modulation of the main process parameter (temperature and humidity); the diffusion test consists of making a dot series by leaving the tip in contact with surface for different times, and then measuring their area using the Nanoink® Inkcal software. The image of the generated dots was achieved as in the writing tests making a scan at 5 Hz (figure 1).

In order to investigate the oligonucleotide-polymer diffusion process at the nanometer level, several deposition experiments were carried out for each ink – substrate pair varying at first the temperature with constant relative humidity, then doing the opposite. Each test consisted in the deposition of a row of seven spots with different dwell times.

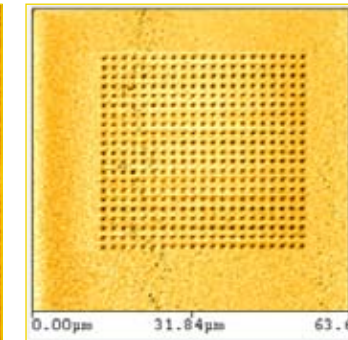
This was carried out keeping the relative humidity constant at 26% with progressive variation of the temperature from 20°C to 30°C increasing the temperature by two degrees each time. The same tests were then repeated keeping the temperature at 24°C and increasing the relative humidity from 5% to 40% (5% 10% 20% 30% 40%).

The next step was to try and obtain high-density nanoarrays with a large number of spots. The pair made of 23-mer acrylamide-modified oligonucleotide and PMMA were selected because of the optimal quality of their images. Figure 2 shows the AFM analysis of the obtained nanoarray.

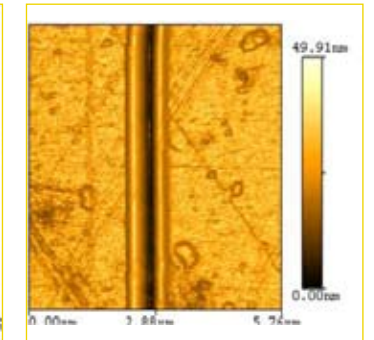
The array consists of 441 spots (21x21) with a diameter smaller than 200nm. The dwell time for the deposition of each spot is 0.2 sec, which means that the whole array was deposited in about 88 sec. Finally, we explored also the hybridation reaction with the complementary oligonucleotide through fluorescence microscopy. The further development and strengthening of such technique would allow the simultaneous analysis of several genes, working with small volumes, reduced times and generating information on the nucleotidic acids sequencing in a way that is faster, easier and cheaper than the traditional ones.



1. Calibration test performed writing dots at increasing contact times and calibration curve



2. High spot density nanoarray



3. Topographic images of nanoscratch analysis performed on epoxy resins coatings

## Mechanical Nanowriting

Thin films coatings find wide application/use in different fields, such as anticorrosion, tribology, electronics. Thin film hardness evaluation is quite complicated due to their very low thickness. Mechanical nanowriting is a technique which makes possible the coating surface hardness measurement without any interference of the substrate beneath. Such technique performs scratch lines using AFM tip onto the to-be analyzed surface. Especially, the tip, to which is applied a constant and known load, is put in contact with the surface and move in a certain direction. During this tip motion, the scratch line on the surface is generated. In figure 3 is reported a AFM image with scratch lines.

Since the applied load and the scratch geometrical features are known and measured, it is been possible to evaluate the film nanoscratch hardness ( $H_s$ ). Assuming as a first approximation a conical shape of the AFM tip, the scratch hardness  $H_s$  may be defined as follows:

$$H_s = \frac{4F}{\pi w^2}$$

where  $F$  is the normal load applied on the AFM tip and  $w$  is the width of the scratch. Test coatings have been prepared both through crosslinking reactions: the first one between epoxy resins based on the bis-glycidil ether of bisphenol A (DGEBA) as component A and diamine hardener (isophorone diamine) diluted with benzyl alcohol as

component B, and second one with sol-gel chemistry (organic/inorganic hybrids).

# ENGINEERED HYDROGEL CONSTRUCTS FOR DRUG DELIVERY AND CELL HOUSING IN CENTRAL NERVOUS SYSTEM APPLICATIONS

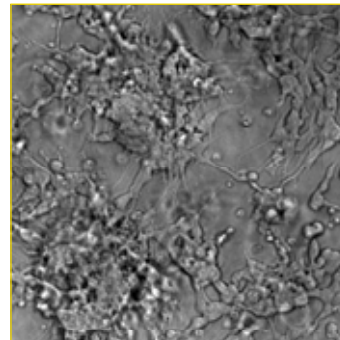
Marta Tunesi

This project focused on the preparation and characterization of hydrogel-based systems for drug delivery and cell conveying in regenerative or neuroprotective approaches for central nervous system degenerative disorders. In particular, Parkinson's disease, Alzheimer's disease and ischemic stroke were considered and different strategies were proposed to suggest innovative way of treatment.

Since in Parkinson's and Alzheimer's diseases some proteins are known to play key roles, the first proposed approach is based on the design and development of suitable polymer matrices for recombinant 70kDa molecular weight heat shock protein controlled delivery, as cast (drug delivery-based strategy) or thanks to engineered cells, acting as drug reservoirs (cell-based drug delivery strategy). Both for *in situ* protein release and *in situ* cell conveying, injectable and biodegradable hydrogel-based systems are appealing and minimally invasive tools to be exploited. In fact after loading with the suitable drug or cells, they may be placed into target volumes without highly risky surgical procedures and they do not require additional surgeries to recover the exhausted implant. In collaboration with Institute of Composite and Biomedical

Materials (National Research Council, Università degli Studi di Napoli "Federico II", Ing. Gloria) in Naples and "Mario Negri" Institute for Pharmacological Research (Dott. Albani) in Milan, two collagen and poly(ethylene glycol)-based semi-interpenetrating networks differing from the volumetric ratio between the component solutions were investigated. A rheological characterization was performed at Institute of Composite and Biomedical Materials: material elastic and loss moduli were evaluated as functions of frequency by small amplitude oscillatory shear tests, their viscosity was examined as a function of shear rate by steady shear measurements and their injectability was confirmed with an INSTRON 5566 testing machine for the same configuration later applied to *in vivo* intrastriatal injections; furthermore for cell-loaded samples, morphology was observed with a transmission electron microscope at Università degli Studi dell'Insubria (Varese).

In order to assess cell response to developed materials, *in vitro* studies were performed with a suitable neuroblastoma cell line (Figure 1); in particular after culturing within hydrogels cell viability was tested at different time points by direct counting after live/dead staining following



1. Neuroblastoma cell line cultured for 7 days within one of tested collagen and poly(ethylene glycol)-based semi-interpenetrating networks. Scale bar = 50  $\mu$ m

extraction from materials and while embedded by a spectrophotometric assay.

For the stiffest matrix, that is the one with the highest collagen content, further *in vitro* studies were carried out with more sensitive cell models, that are mesencephalic neurons from mouse embryos and undifferentiated rat mesenchymal stem cells. For this composition *in vivo* tests were also performed: at "Mario Negri" Institute for Pharmacological Research peripheral inflammatory reactions were investigated after injection in mouse subcutaneous dorsal pouches by leukocyte counting, while after mouse intrastriatal injection brain cytoarchitecture and degenerating synapses were stained by Nissl and Fluoro-Jade staining (respectively) and finally

at Prof. Bizzi Lab at McGovern Institute for Brain Research at Massachusetts Institute of Technology rats were checked for macroscopic motor deficits after injection in motor cortex under different surgical conditions. With regards to protein release, a recombinant form of 70kDa heat shock protein (kindly synthesized by Prof. Negro and colleagues, University of Padua) was loaded within the selected hydrogel formulation and its release was followed with time by Western Blotting.

Concerning ischemic stroke, a novel set of hydrogel formulations based on agarose (A) and Carbomer 974P (C) as main components were developed and they were included in a Politecnico di Milano patent. Since it is known to promote neural precursor cell proliferation and differentiation, hydrogels were then modified by adding poly(ethylene glycol) to two selected formulations differing from (m/m) A/C ratio. Because of A interesting potential in cell housing applications, matrices with the highest (m/m) A/C ratios were considered for cell conveying-based approaches.

For drug delivery-based applications, six compositions were tested. Material swelling behaviour was evaluated with time for freeze-dried samples

in different media commonly used for *in vitro* experiments (cell culture medium, phosphate buffered saline solution and water at different pHs) to assess the effect of their composition and pH on material behaviour and account for inflammatory processes, which are known to modify local *in vivo* pH values. In order to evaluate cell ability to survive the period of latency within matrices, *in vitro* cell response was assessed with a suitable glioblastoma-astrocytoma cell line by live/dead staining following extraction from matrices and by a spectrophotometric assay after replating. A preliminary *in vitro* release study was then carried out with 17 $\beta$ -estradiol (a well known neuroprotective estrogen), a mathematic model based on diffusion only was proposed to fit experimental data and a setup to measure the diffusion coefficient for solutes diffusing through hydrogels was preliminarily tested.

For cell conveying-based applications, six compositions were also examined. Hydrogel swelling behaviour was evaluated with time in phosphate buffered saline solution and cell culture medium, reproducing cell embedding conditions. In collaboration with Neuro-Zone s.r.l. (Dott. Bianco) in Milan, *in vitro* biocompatibility

was assessed with suitable glioblastoma-astrocytoma and microglial cell lines by live/dead staining following extraction from matrices; furthermore a preliminary functional test was performed for microglial cells after replating by single cell calcium level recording. Since obtaining pure neural progenitor cell cultures is difficult, preliminary investigations with stem cells were carried out with undifferentiated rat mesenchymal stem cells, whose viability was assessed by live/dead staining after extraction from hydrogels.