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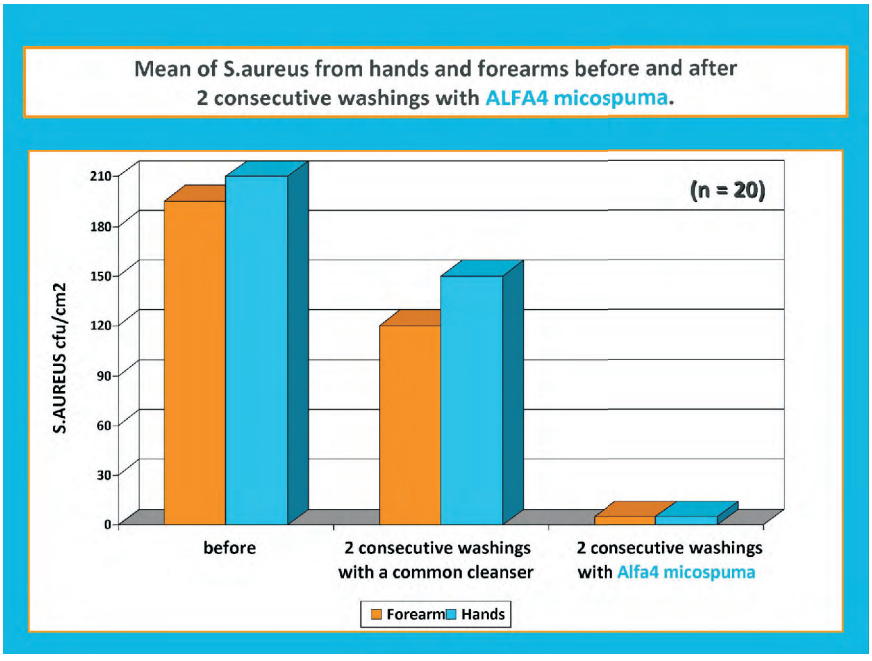

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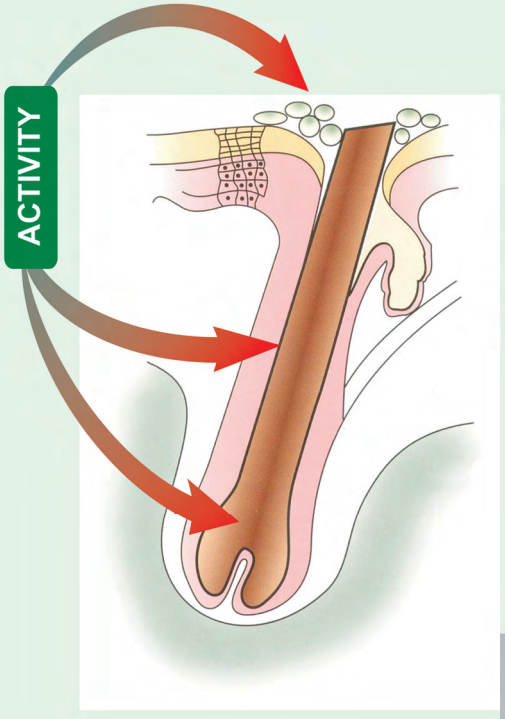

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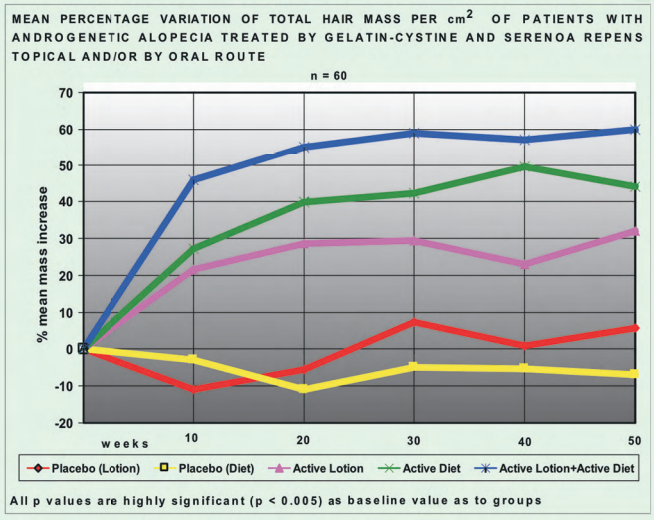

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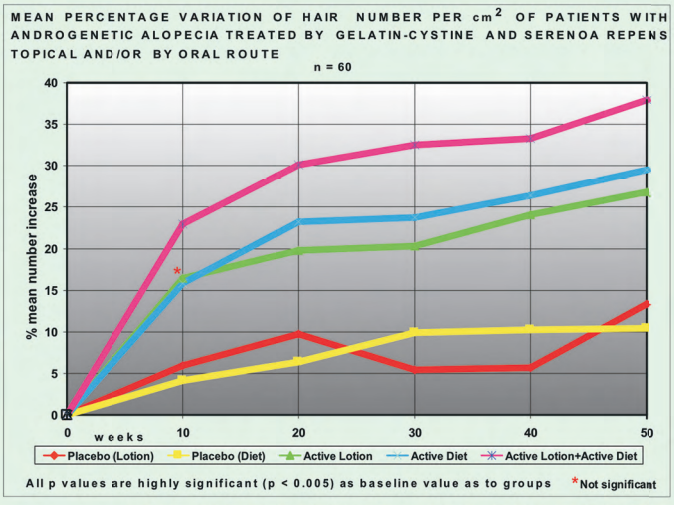

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(1) Morganti P, Fabrizi G, James B, Bruno C, *J. Appl. Cosmetol.***16**,57,1998

(2) Fabrizi G, Morganti P, (1999),*SÖFW-Journal*, **125**, 2/3 :10-13

(3) Morganti P, (1999),*Eurocosmetics* **9**: 30-32

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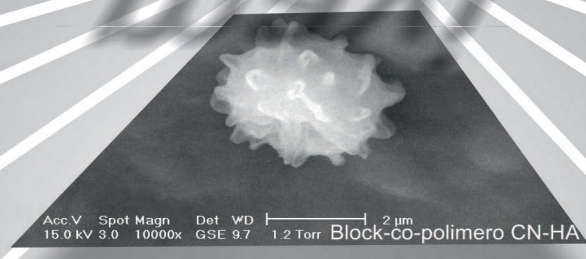

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3

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Trimestrale di Dermatologia Cosmetologica
Quarterly Review of Cosmetic Dermatology

Contents

Bioresorption of porous three-dimensional chitosan-based materials intended for use in medical surgical cosmetology and tissue engineering

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Key words: *3D porous material; Chitosan; Resorption; Tissue engineering; Medical Surgical Cosmetology;*

Summary

Three-dimensional matrices with high porosity were prepared by lyophilization of chitosan solution. The mechanism and rate of resorption of the resulting materials were studied *in vivo*. It was shown that resorption was completed in 12 months after implantation into an animal. The formation of connective tissue was not observed and the surrounding tissue had no signs of changes or damage. Histological analysis demonstrates that chitosan resorption occurred simultaneously with the formation of collagen fibers and blood vessels. These results allow us to recommend the obtained chitosan-based porous material for use as a matrix for tissue engineering and medical cosmetology.

Riassunto

Sono state preparate biometrici tridimensionali e ad alta porosità utilizzando soluzioni di chitosano, studiandone il relativo meccanismo di assorbimento *in vivo*.

È stato verificato come dopo 12 mesi di impianto sull'animale, le biometrici vengano completamente riassorbite. Non è stata osservata formazione di tessuto connettivo, né sono apparsi segni di cambiamento nei tessuti o danni.

Le analisi istologiche dimostrano come il riassorbimento del chitosano avvenga simultaneamente alla formazione delle fibre di collageno dei vasi sanguigni. Tali risultati pongono in evidenza e raccomandano l'uso di questo materiale poroso come matrice per l'ingegneria e la riorganizzazione dei tessuti.

Abbreviations: PCM — porous chitosan matrix.

INTRODUCTION

Intensive development of medical surgical cosmetology and tissue engineering requires designing bioengineering preparations that consist of a polymer matrix and stem or somatic cells. These preparations should serve as functional substitutes for lost organs or their parts, and should not cause autoimmune rejection. The development of bioengineering preparations allows also abandoning the use of donor tissues and organs which are necessary in modern transplantology.

The matrix material and its structure should facilitate adhesion, proliferation and differentiation of recipient cells. To achieve this, the matrix should possess a certain combination of properties (biocompatibility, porosity, absence of toxicity, relatively high mechanical strength and elasticity that are necessary for manipulations in liquid media (1-3)).

The polymers using for process of bioresorbable matrices are polylactide, polyglycolide, polycaprolactone, polysaccharides, collagen and some other polymers (1). Chitosan is one of the most promising polymers with the necessary complex of properties (4-6); its macromolecules consist of randomly arranged β-D-glucosamine and N-acetyl-β-D-glucosamine units. Chitosan is a bioresorbable polymer which demonstrates antibacterial, antiviral and fungicide activity (7-9), and thus it can be considered as a promising material for applications in medicine, biology, pharmaceutics and other areas. The useful properties of chitosan are related to its chemical structure; due to the presence of a large amount of free amino groups in chitosan molecule, it can bind protons and acquire excess positive charge. Thus, chitosan is an excellent cationite.

In human or animal organism, chitosan is decomposed into N-acetyl-β-D-glucosamine and β-D-glucosamine (10) that is the natural components of intercellular matrix, synovial liquid and cartilaginous tissue and are included into the hyaluronic acid glycosaminoglycan. Chitosan is used for preparation of films, fibers and bulk porous materials (sponges) by the following methods: coagulation of polymer solution in a precipitant, drying or sublimation of a solvent etc. (11-14).

For the tasks of medical cosmetology, tissue engineering and transplantology, it is necessary to obtain three-dimensional porous matrices (prototypes of bone and parenchymatous tissues). After implantation of a tissue engineering material into a living organism, two simultaneous processes occur, that is, a cellular process (migration of cells into the matrix, their proliferation and differentiation) and resorption of the polymer matrix. Successful replacement of natural tissue is possible only at a certain ratio between rates of these processes. Resorption of matrix should not lead cell migration and proliferation. It should be noted that destruction of matrix material under the action of the active biological medium is a complex process that depends on many factors.

Analysis of the literature showed that rate of matrix resorption depends on molecular mass of a polymer (15), porosity of a material (16), the presence of nanoparticles and other polymers in the matrix material (17). In (13), the authors have studied resorption of chitosan fibers in endomysium and perimysium of the m. latissimus dorsi of rat; it has been demonstrated that applying dynamical mechanical loads and intensive metabolic processes in muscle endomysium lead to a considerable increase in the rate of chitosan fiber resorption. Other way, in the absence of listed above processes, fiber implant becomes encapsulated. It is important to note that since the process is influenced by many factors, it is very difficult to simulate bioresorption of a polymer matrix *in vitro*. Therefore, *in vivo* studies of mechanism and kinetics of polymer matrix resorption are very important for successful solving problems of medical cosmetology, tissue engineering and

transplantology.

The aim of the present work was the *in vivo* study of resorption of three-dimensional porous materials made of chitosan in a rat muscle tissue.

MATERIALS AND METHODS

Preparation of the matrices

Porous chitosan matrices (PCM) were prepared from the chitosan sample purchased from Sigma-Aldrich Corporation (USA) with a molecular mass of 200 kDa and a deacetylation degree of 80%.

Chitosan was dissolved in 2% aqueous solution of acetic acid at continuous stirring for not less than 120 min, concentration of the polymer in solution was 3%. The obtained solutions were filtered, degassed at a pressure of 1 × 105 kPa for 3 hrs, and then were frozen at –20 °C.

The obtained samples were lyophilized at –2°C and a pressure of 1.6 Pa. The lyophilization was performed by using a Freeze Dry System setup (USA). Cylindrical samples 5 mm in length and 1.3 mm in diameter with pore sizes varying from 10 to 300 μm were cut from the obtained block blanks with a tubular cutter. The samples were treated with 5% solution of NaOH in order to transfer chitosan from the water-soluble salt form into the base form that is insoluble in water. Due to its open pore structure, the material possesses a specific surface area of 1 m^2/g, and the water absorption value reaches 3000%.

Scanning electron microscopy images of the samples were obtained with the aid of the Supra 55VP instrument (Carl Zeiss, Germany) using secondary electron imaging. Before the experiments, the samples were coated with a thin layer of platinum.

Experiments on animals

The *in vivo* experiments involved 35 male 6-month-old Wistar white rats with weights varying from 200 to 250 g. The experiments were carried out according to the principles of the European Convention (Strasbourg, 1986) and the Helsinki Declaration of the World Medical Association about humane treatment of animals (1996).

Cylindrical chitosan samples used in the *in vivo* studies of resorption were sterilized in the wet state by autoclave at 120°C for 40 min. The animals were operated under general anesthesia (Zoletil 100 solution (0.1 mL, per 0.1 kg animal body mass) and Rometar 20 mg/mL (0.0125 mL per 0.1 kg animal body mass), administered intraperitoneally).

The samples were placed into *musculus adductor magnus* of the rat thigh on both extremities; the wounds were stitched up layerwise using atraumatic needles and Prolene 4-0 suture. After suturing, the rats were caged individually, fed standard diet, and had free access to water.

Morphological studies and statistical treatment

Morphological studies were performed in 1, 2, 6, 12, 24, 36 and 48 weeks after the operation; the samples of muscle tissue containing PCM were fixed in 10% neutral phosphate-buffered formalin (pH = 7.4) for not less than 24 hrs, dehydrated in a series of ethanol solutions of increasing concentrations and embedded in paraffin blocks according to the standard histological technique. Transverse paraffin sections of muscle fibers with implanted PCM 5 μm thick were stained with Karazi's hematoxylin and eosin (Bio-Optica, Italy).

Connective tissue was visualized with Mallory's hematoxylin (Bio-Optica, Italy). Microscopic analysis was carried out using a Leica DM750 light microscope (Germany), ocular ×10, lenses ×4, 10, 40, 100. Photographs were taken with an ICC50 camera (Leica, Germany).

Morphometric analysis of muscle tissue samples was performed by stereologic point counting with the use of a test mesh of ocular micrometer with 25 points (ocular ×7, lens ×40). The data were obtained after registration of 1000 points (this amount was taken as 100%). From the results, the relative volumes occupied by leukocytes, gigantic multinuclear cells, fibroblasts, vessels, and collagen in pores of the matrix, and the relative volume occupied by the matrix structure were determined. The total area of transverse PCM section was calculated with the use of the "Video Test" software (ocular ×7, lens ×40).
Statistical analysis of the obtained results was performed with the use of a statistical program for Windows (Statistica 7.0 Stat.Soft). The significance of differences was estimated using the Mann-Whitney U test. The differences were considered significant at $P \leq 0.05$.

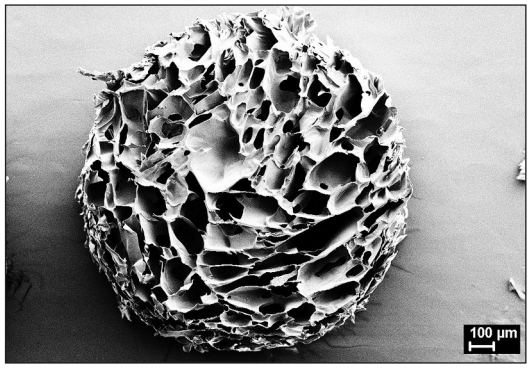
RESULTS AND DISCUSSION

The Figure 1 presents microphotographs of the surface of a cross-section of the chitosan-based material. It can be seen that the material contains a system of open pores that are connected with each other and with external environment.

This structure allows for nutrients, waste products and dissolved gases to circulate freely in the whole matrix. Pore size and insignificant sinuosity of channels that connect pores with each other facilitate free migration of cells.
As it has been demonstrated before (11) with using of dermal fibroblasts and mesenchyme stem cells, the chitosan-based materials do not possess cytotoxicity or genotoxicity at cell levels. So, all animals were active after surgery, no inflammation in the implantation area was observed, which is indicative of the absence of detrimental effects of implantation.

Histological studies of PCM after 1 week of exposure

Histological studies of PCM performed in one week of exposure after implantation revealed mild aseptic inflammation around the matrix with leukocyte infiltration centers and segmented cells prevail in these centers. The matrix is surrounded by plethoric vessels, hemorrhagic centers are observed, and insignificant edema appears around the implant. The matrix cross-section area is equal to 8.16×10^5 μm² (Fig. 2).



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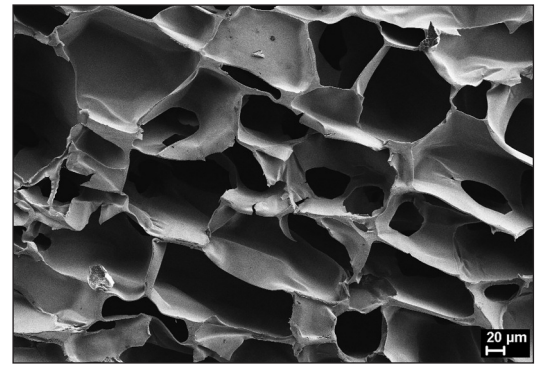


1b

Fig. 1 *Microphotographs of a cross-section of sponge cylinder of PCM samples taken at different magnifications.*

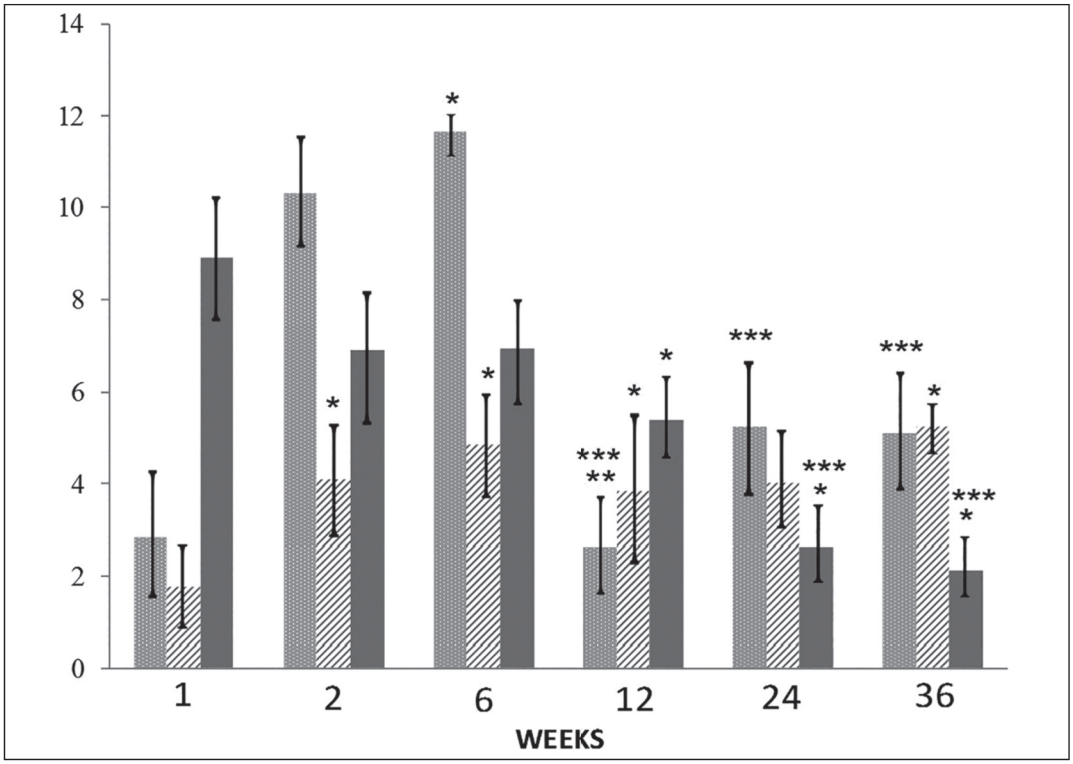


Fig. 2 *anges over time in the PCM cross-section area (dark grey bars, μm²) with respect to the volumes occupied by gigantic cells of foreign bodies (light grey bars, %) and fibroblasts (striped bars, %) occurring in 36 weeks after implantation of the matrix into rat muscle. (Vertical segments represent the values of standard error. The difference between values is statistically significant at P < 0.05 in comparison with the parameters of the 1st week (one asterisk), in comparison with the parameters of the 2nd week (two asterisks) and in comparison with the parameters of the 3rd week (three asterisks) after implantation.).*

TABLE I

The relative volume (%) occupied by biological structures in the matrix cross-section, at different periods after implantation.

Time, weeks	Matrix	Collagen fibers	Vessels	Leukocytes
1	17.7	0.9	0.1	11.9
2	19.1	3.2	0.5	0.9
6	19.4	3.9[a]	0.9	1.2
12	24.7	3.4	0.5	1.6
24	24.9	3.7	1.3[a]	3.9
36	18.7	6.9 [a,b]	0.8	4.6

[a, b] The differences between values are statistically significant in comparison with the values of the 1st and 2nd weeks after implantation (P < 0.05).

The relative volume occupied by the matrix structures is equal to 17.7% (Table I). Gigantic multinuclear cells of foreign bodies are observed around PCM. Some of them penetrate into peripheral pores of the matrix and their relative volume is 2.8%.

In singular peripheral pores, fibroblasts and plethoric vessels are observed (Fig. 3, a, b). The relative volume occupied by fibroblasts is 1.1%, and the relative volume of vessels is 0.1%. The relative volume of collagen in peripheral pores is 0.9% (Table I).

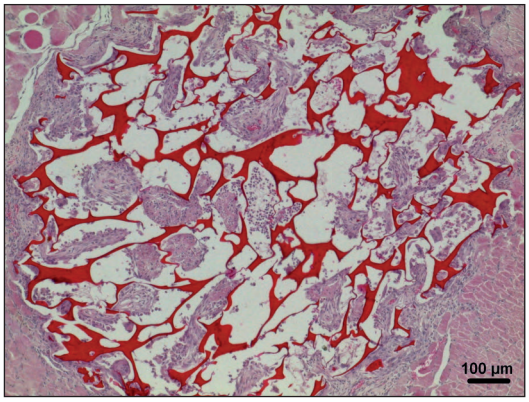
Only fibrin, cellular detritus and infiltrated leukocytes with prevailing segmented cells were revealed in the central pores of PCM (Fig. 3 b). The relative volume of leukocytes in the central pores of the matrix is 11.9% (Table I).

Histological studies of PCM after 2 weeks of exposure

In two weeks after implantation, insignificant aseptic inflammation was observed around PCM, but the connective tissue capsule and edema were not formed. The cross-section area of the matrix slightly decreases and is equal to 6.88×10^5 μm^2 (84% from the corresponding value obtained in 1 week after implantation, Fig. 2). The relative volume of the matrix does not change (Fig. 3, c). PCM pores are filled with gigantic multinuclear cells (Fig. 3, d), and their relative volume increases up to 10.3% (i.e., comprises 364% of the initial value). In the majority of samples, the formed connective tissue with fibroblasts fills virtually all pores, but in one case, the central pores are still filled with leukocytes (Fig. 3, c). Plethoric vessels are observed in several pores. The relative volume of components of connective tissue that fills PCM increases, it is equal to 5.1% for fibroblasts. This value differs significantly from the corresponding parameter obtained in 1 week after implantation (the increase is 475% with respect to the value obtained in 1 week). The relative volume of vessels is 0.5% (400% of the value obtained in 1 week) and the relative volume of collagen is 3.2% (344% of the value obtained in 1 week). Leukocytes (mainly, lymphocytes) are observed both in central and peripheral pores of the matrix. The relative volume of leukocytes decreases down to 0.9% (7% of the corresponding value obtained in 1 week after implantation (Table I, Fig. 2)).

Histological studies of PCM after 6 weeks of exposure

In six weeks after operation, no connective tissue capsule was revealed around PCM (Fig. 3, e). The cross-section of the matrix continues to decrease insignificantly and becomes equal to 6.38×10^5 μm^2 (78% of the value obtained in 1 week after implantation) (Fig. 2). The relative volume of the matrix is 19.4%, and this value does not significantly differ from the values obtained in 1 and 2 weeks after implantation. All pores are filled with connective tissue which contains fibroblasts and gigantic multinuclear cells (Fig. 3, f). Plethoric vessels were found in many pores. It should be noted that the relative volume of fibroblasts increases significantly up to 4.9% (450% of the value obtained in 1 week after implantation). The relative value of collagen (3.9%, 419% of the level registered in 1 week) and vessels (0.9%, 733% of the level registered in 1 week) also increase significantly. In this period, the relative volume of gigantic multinuclear cells also increases and differs significantly from the corresponding parameter in 1 week after implantation. In the pores of matrix, mainly lymphocytes are observed. Their relative volume is 1.2%, which does not differ significantly from the value of the 1st week (Table I, Fig. 2).



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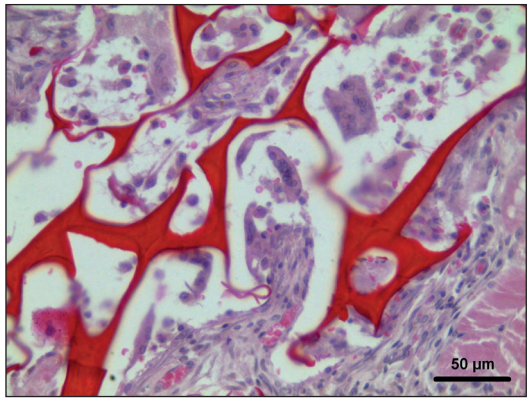

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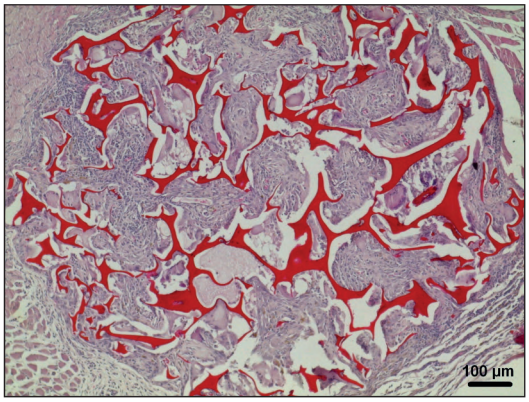

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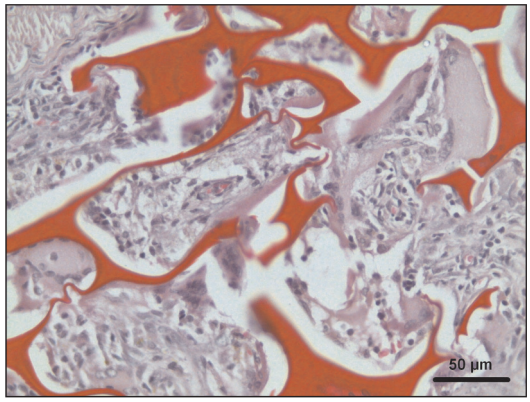

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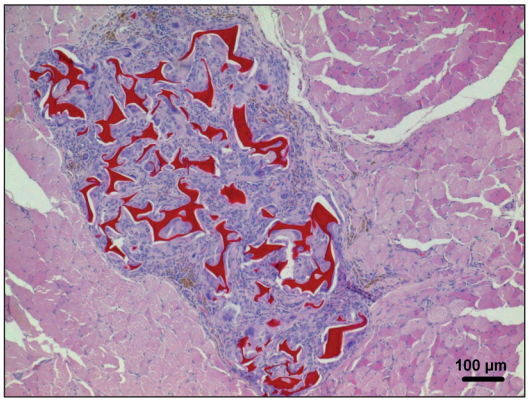

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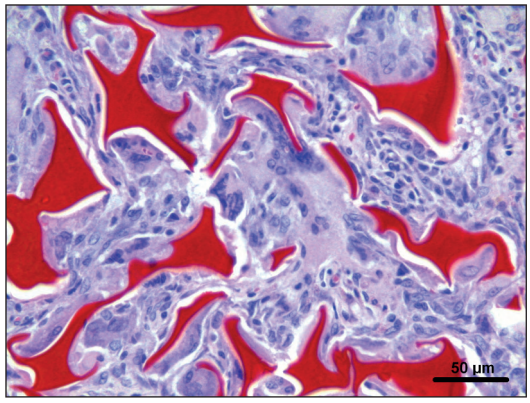

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Fig. 3 *Histological sections of rat muscle tissue obtained in 1 (a, b), 2 (c, d) and 6 (e, f) weeks of PCM exposure. The samples were stained with hematoxylin and eosin; (a, c, e): objective magnification ×10; (b, d, f): objective magnification ×40.*

Histological studies of PCM after 12 weeks of exposure

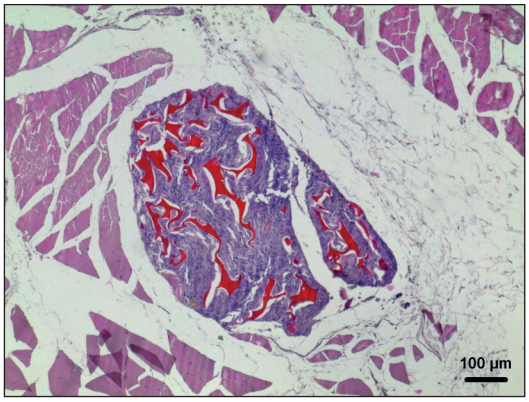
In 12 weeks after implantation (Fig. 2), the cross-section area of the matrix decreases down to 5.38×10^5 μm^2, which differs from the respective parameter obtained in 1 week after implantation (65.9%). There is no connective tissue capsule around the implant. The signs of chronic proliferative inflammation appear; accumulations of lymphocytes are observed as well as macrophage and histiocyte cells. The relative volume of matrix structures is 24.7%, which does not differ significantly from the values obtained in the previous periods (139% of the value of the 1st week). All pores of the matrix are filled with connective tissue. The relative volume of fibroblasts is 3.8% and, similarly to the previous periods, differs from the value of the 1st week (355%). The amount of multinuclear gigantic cells decreases sharply and reaches 2.6% and this value differs reliably from the respective parameters of the 2nd and 6th weeks after implantation. The relative volumes of collagen fibers, vessels and leukocytes do not change significantly (Table I, Fig. 2). However, it should be noted that unlike the earlier period of the experiment, lymphocytes now prevail and form small clusters in some pores.

Histological studies of PCM after 24 weeks of exposure

In 24 weeks after PCM implantation, the cross-section area of the matrix decreases down to 2.64×10^5 μm^2, which differs reliably from the values of the 1st and 6th weeks (it decreases by 32.3% as compared to the value of the 1st week, Fig. 2). There is no connective tissue capsule around the implant (Fig. 4, a), and the signs of chronic proliferative inflammation are still present. The relative volume of the matrix does not change. All pores of the matrix contain fibroblasts and leukocytes with lymphocytes predominating; their relative volume does not change significantly in comparison with the respective parameters obtained in the early periods of the experiment (Fig. 4, b). The volume of multinuclear cells increases up to 5.3% and differs from the values of the 6th week after implantation (the increase is 187% as compared to the value of the 1st week). The relative volume of collagen fibers also does not change, while the relative volume of vessels increases up to 1.3%. This value differs reliably from the value of the 1st week (Table I, Fig. 2).

Histological studies of PCM after 36 weeks of exposure

In 36 weeks after implantation (Fig. 4, c, d, e), the cross-section area of the matrix continues to decrease and reaches 2.14×10^5 μm^2, which differs reliably from the values of the 1st and 6th weeks. There is no connective tissue capsule around PCM (Fig. 4, c, d). The relative volume of the matrix structures does not differ significantly from the values of the early periods. The relative volumes of fibroblasts and gigantic multinuclear cells remain virtually the same, and the volume of collagen fibers increases up to 6.9%, which differs from the values of the 1st and 2nd weeks (734% with respect to the value obtained in the 1st week). The relative volumes of vessels and leukocytes do not change noticeably in comparison with those registered in the early periods after implantation (Table I, Fig. 2).



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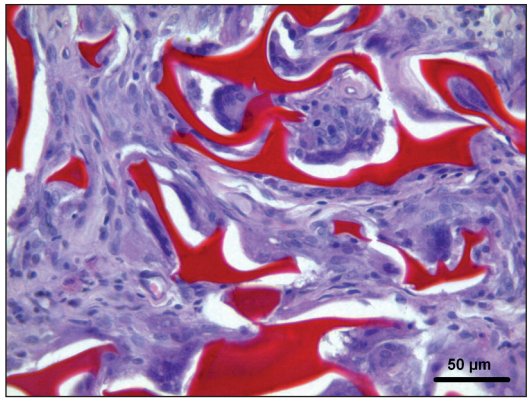

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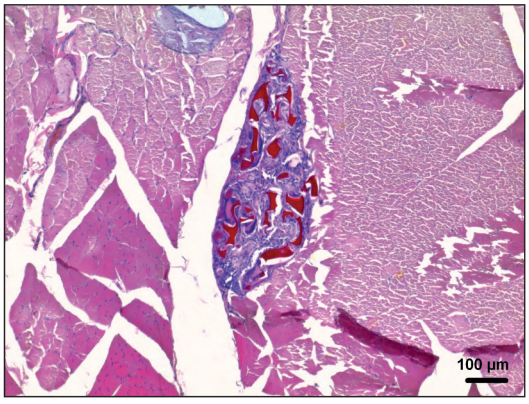

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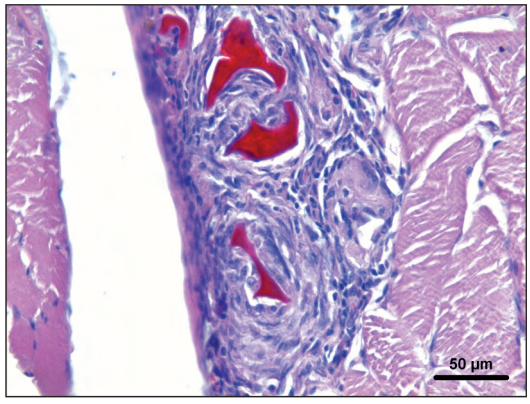

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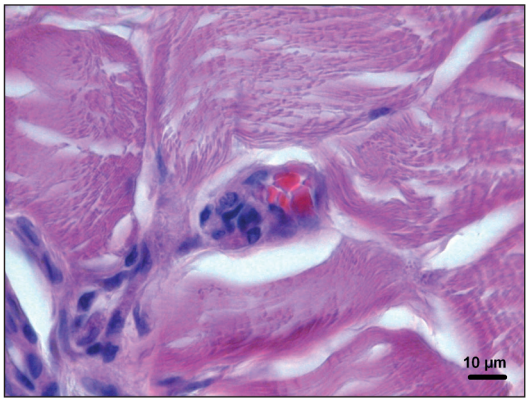

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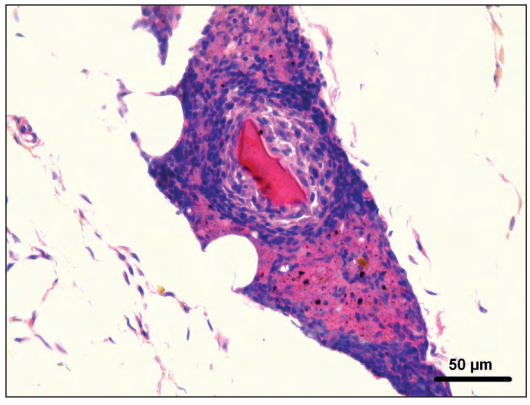

4f

Fig. 4 *Histological sections of muscle tissue with PCM obtained in 24 (a, b), 36 (c, d, e) and 48 (f) weeks of in vivo exposure. The samples were stained with hematoxylin and eosin; (a, c): objective magnification ×10; (b, d, f): objective magnification ×40; (e): objective magnification ×100.*

Histological studies of PCM after 48 weeks of exposure

In 48 weeks after the beginning of the experiment, fragments of PCM were found only in one of 5 tissue samples (Fig. 4, f). The cross-section area of the matrix decreases down to 1.71×10^5 μm^2. Connective tissue capsule is not observed, and the matrix structures which are not connected to each other were found around the fragments; the signs of chronic aseptic proliferative inflammation are present. PCM fragments are surrounded by macrophages and histiocytes followed by a layer of lymphocytes, and fibroblasts are located at the periphery.

CONCLUSION

It was demonstrated that complete bioresorption of PCM in muscle tissue occurs in 48 weeks after implantation, no connective tissue is formed and the surrounding tissues are not damaged or changed. Throughout the experiment, the matrix retained its shape and the pores remained open, these properties facilitated vascular invasion and free migration of cells in the bulk of the matrix. The resorption of the matrix occurred simultaneously with the process of filling PCM with cellular structures. The results allow us to recommend PCM for manufacturing bioengineering preparations that can be used in medical surgical cosmetology and tissue engineering

ACKNOWLEDGEMENTS

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Molecular docking analysis of Alginate oligosaccharides (Alg2-Alg6) as bacterial collagenase inhibitor

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Key words: *Molecular docking; Alginate oligosaccharides; Clostridium histolyticum collagenase inhibitor; L-cysteine; N-Acetyl-L-cysteine.*

Summary

In recent years, marine oligosaccharides have gained much importance especially for their various biological applications. Alginate oligosaccharides, obtained through enzymatic hydrolytic method, have been reported to show anti-oxidant and anti-inflammatory activities. The present study describes molecular docking analysis of alginate oligosaccharides (Alg2-Alg6) as bacterial collagenase inhibitor. Alginate oligosaccharides (Alg2-Alg6) were evaluated on the docking behaviour of bacterial collagenase using PatchDock. In addition to this, ADME (Absorption, Distribution, Metabolism and Excretion) analysis was done. The docking studies and binding site analyses revealed that Alg5 (M-G-M-G-M residue) with the highest ACE (*Atomic Contact Energy*, -183.29 kcal/mol) and AlgM (M residue alone) give the least Atomic Contact Energy -1.18 kcal/mol) of *Clostridium histolyticum* collagenase. Interestingly, Alg2 (M-G residue) has shown to interact with Tyr618 and Asp737 amino acid residue of *C. histolyticum* collagenase. Thus, the results of the present study exhibited the potential of these alginate oligosaccharides (Alg2-Alg6) as bacterial collagenase inhibitory agent.

Riassunto

In questi ultimi anni l'uso degli oligosaccaridi di origine marina ha assunto molta importanza per diverse applicazioni biologiche.
Con questo studio è stato posto in evidenza che gli alginati ottenuti per idrolisi enzimatica svolgono interessanti attività antiossidante ed antinfiammatoria. Lo studio riporta l'analisi di aggancio molecolare degli alginati Alg2-Alg6 come inibitori delle collagenasi batteriche ottenuta con l'uso del

PatchDock. È stato anche verificato il grado di assorbimento, distribuzione, metabolismo ed escrezione (ADME) del polisaccaride in studio.
Gli studi di aggancio e di legame hanno posto in evidenza come Alg5 (il residuo M-G-M-G-M) con il più alto ACE (-183.29 Kcal/Mol) e AlgM (il solo residuo M) rivelano di avere un minimo ACE (-1.18 Kcal/Mol) relativamente alla collagenasi del *Clostridium histolyticum*. È interessante osservare come AlG2 (il residuo M-G) interagisca con i residui amminoacidici Tyr618 e Asp737 di questa collagenasi.
I risultati ottenuti mettono in evidenza il potenziale uso di questi alginati (Alg2-Alg6) come agenti inibitori delle collagenasi batteriche (ACE= *Atomic Contact Energy*).

INTRODUCTION

Microbial collagenases, belong to the MEROPS peptidase family M9, which consists of bacterial metalloproteinases from *Vibrio* and *Clostridium* species show collagenolytic activity (1). These enzymes well known as virulence factors of many pathogenic bacteria, serve as potential targets for antibiotic drug discovery (2). Collagenases have been reported in many species from *Clostridium* such as *C. botulinum, C. difficile, C. histolyticum, C. perfringens* and *C. tetani*. These enzymes have been used as main proteolytic enzyme source for medicinal and industrial purposes (3). Similarly, the anti-collagenase activity of several natural products has been studied using *Clostridium histolyticum* collagenase as enzyme source (4) as well as its inhibitors have been developed for treating bacterial corneal keratitis disease (5).

Alginate is an anionic polysaccharide found in the brown seaweed or algae. It is composed of alpha-L-guluronate (G) and beta-D-mannuronate (M) residues arranged in a block structure as a homopolymer (poly-G/poly-M) or heteropolymer (a mixed sequence of both the G & M residues). Commercially available alginate is extracted from brown algae such as *Ascophyllum nodosum, Laminaria digitata, Laminaria hyperborea, Laminaria japonica* and *Macrocystis pyrifera* (6). It has been reported as biocompatible, mucoadhesive, non-immunogenic substance which undergoes dissolution and biodegradation under normal physiological conditions (7). Alginate is widely used as biomaterial especially for tissue repair and regeneration, as well as drug and protein delivery agent. It has been approved as biopolymer material by United States (US) Food and Drug Administration (FDA) agency (8). Alginate has been used as wound dressing materials, where Algicell™ , Algisite™ , Comfeel plus™ , Kalto stat™ , Sorbsan™ and Tegagen ™ are examples of few commercially available alginate-based wound dressing products (9). This prompted us to carry out the docking study on alginate oligosaccharides (Alg2-Alg6) against bacterial collagenase. Alginate oligosaccharides (Alg2-Alg6) were evaluated on the docking behaviour of bacterial collagenase using PatchDock. In addition, ADME (Absorption, Distribution, Metabolism and Excretion) analysis of all the ligands using SwissADME was carried out.

MATERIALS AND METHODS

Ligand preparation

Chemical structures of the ligands namely I) L-cysteine [CID5862] and II) N-Acetyl-L-cysteine [CID12035] were retrieved from PubMed (www. pubmed.com). The unavailable ligands (namely M residue, alginate oligosaccharides Alg2-Alg6) were drawn in ChemBioDraw Ultra 12.0 (www.cambridgesoft.com) while molecular mechanic (MM2) minimization of ligands were carried out by ChemBio3D Ultra 12.0. Thus, these structures were employed for further PatchDock.

Target protein identification and preparation

The three dimensional (3D) structure of the *Clostridium histolyticum* collagenase (PDB ID: 4ARE with resolution of 2.19 Å) was obtained from the Research Collaborator for Structural Bioinformatics (RCSB) Protein Data Bank (www.rcsb.org). A chain of the proteins was preprocessed separately by deleting ligands, as well as the crystallographically water molecules (water without hydrogen bonds) were observed. The protein mentioned above was prepared using UCSF Chimera software (www.cgi.ucsf.edu/chimera).

ADME analysis

ADME (Absorption, Distribution, Metabolism and Excretion) analysis was performed by SwissADME (www.swissadme.ch) online tool and ADME analysis was carried by a standard default protocol (10).

Docking studies

Docking studies were carried out by the PatchDock online server (http://bioinfo3d.cs.tau.ac.il/PatchDock). PatchDock adopts geometry based molecular docking algorithm method was used to recognize the binding scores, by binding residues atomic contact energy of the given ligands (11). The docking results were obtained through the user email address. We also use to get uniform resource locator (URL) which provides the top 20 solutions in a table form via email. From these, the top one solution (the docked protein-ligand complex) was selected and downloaded in a program database (pdb) file format. Further, the binding site analyses were carried by PyMOL software (www.pymol.org).

RESULTS AND DISCUSSION

Marine fauna and flora has been used as source of novel drugs, showing a rapid growth in the recent years. Alginate is an anionic biopolymer which has been widely used in the various fields such as biomedical, cosmetics and pharmaceutical applications due to its biocompatibility and other favourable properties. It has been used as thickening and stabilizing agent of the oil phase, in emulsifier free cosmetic formulations. Chitosan-treated alginate micro particles, loaded with all-trans retinoic acid, have shown to enhance dermal localization as well as sustain release of all-trans retinoic acid into the skin (12). Similarly pectin treated alginate microspheres have shown to exhibit the sustained release of Vitamin E in cosmetic formulations (13). Thus, alginate used as wound dressing materials bound to silver, has shown to enhance both antimicrobial and antioxidant activities. Moreover, it exhibited a binding affinity towards elastase, matrix metalloproteinases 2 (MMP 2) and proinflammatory cytokines (14). In addition, alginate has been reported to have also anticancer activity (15). Recently Kelishomi and coworkers (16) reported that low molecular weight alginate (LMWA) show to have antioxidant activity as well as oligosaccharide obtained through enzymatic treatment showed antioxidant (17) and anti-inflammatory (18) activities. Matrix metalloproteinase's or gelatinase's (MMP 2 and MMP 9) levels are elevated in the UVB induced skin, which leads to wrinkle formation by the destruction of basement membrane structure and dermal collagen. Thus, topical application of MMPs inhibitors may represent a solution to overcome this problem (19).

In the present study, alginate oligosaccharides (Alg2-Alg6) were evaluated on the docking behaviour of *Clostridium histolyticum* collagenase using PatchDock.

Two dimensional structure (2D) of AlgM (M residue alone), Alginate oligosaccharides (Alg2-Alg6), L-cysteine and N-Acetyl-L-cysteine were represented in the Figure 1.

However, it is better to know the ADME profile of alginate oligosaccharides (Alg2-Alg6), before performing PatchDock. Thus, table I shows the ADME profile, where all the alginate oligosaccharides (Alg2-Alg6), are predicated to have low gastrointestinal (GI) absorption effect, whereas AlgM (M residue alone), L-cysteine and N-Acetyl-L-cysteine (NAC) are predicated to have high gastrointestinal (GI) absorption effect and P-glycoprotein (P-gp) is one of the main obstacles for delivering drug properly (20). In the present study AlgM (M residue alone) and Alginate oligosaccharides (Alg2-Alg6) are predicated to have P-gp substrate property.

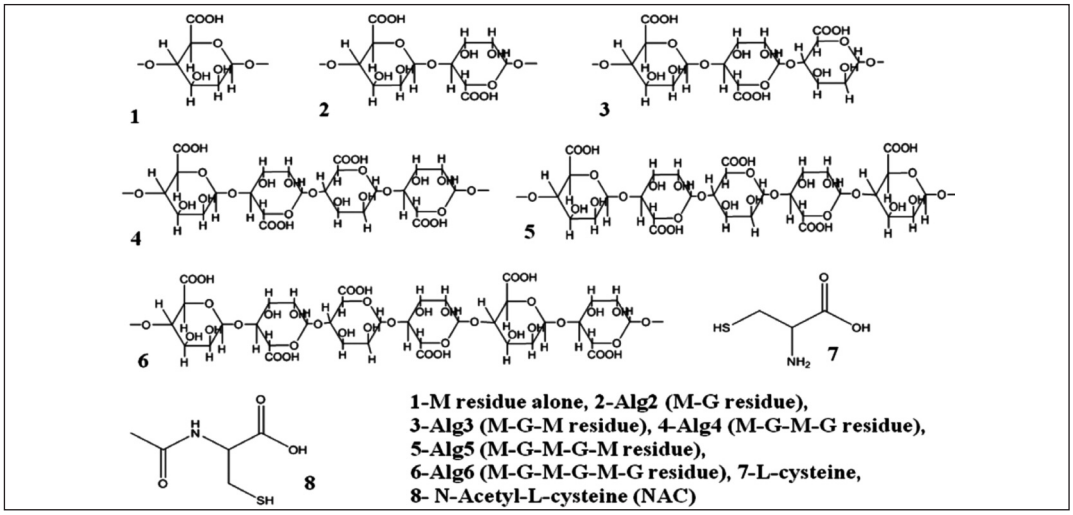

Fig. 1 *Represent two dimensional structure of Alginate oligosaccharides (Alg2-Alg6), L-cysteine and N-Acetyl-L-cysteine.*

TABLE I

ADME analysis of Alginate oligosaccharides (Alg2-Alg6), L-cysteine and N-Acetyl-L-cysteine (NAC) using SwissADME online tool.

Ligand	GI•	BBB▪	P-gp□	CYP1 A2*	CYP2 C19*	CYP2 C9*	CYP2 D6*	CYP 3A4*	Log Kp◊
AlgM (M residue alone)	High	No	Yes	No	No	No	No	No	-8.95
Alg2 (M-G residue)	Low	No	Yes	No	No	No	No	No	-11.37
Alg3 (M-G-M residue)	Low	No	Yes	No	No	No	No	No	-13.79
Alg4(M-G-M-G residue)	Low	No	Yes	No	No	No	No	No	-16.21
Alg5 (M-G-M-G-M residue)	Low	No	Yes	No	No	No	No	No	-18.63
Alg6 (M-G-M-G-M-G residue)	Low	No	Yes	No	No	No	No	No	-21.06
L-Cysteine	High	No	No	No	No	No	No	No	-8.81
N-Acetyl-L-Cysteine (NAC)	High	No	No	No	No	No	No	No	-7.04

•- GI-Gastrointestinal absorption, ▪- BBB-Blood brain barrier permeant, □- P-gp- P-glycoprotein substrate, CYP - Cytochrome P450, *- Inhibitor, ◊- Skin permeation (cm/s).

The docking studies and binding site, analyzed in Table II, shows that Alg5 (M-G-M-G-M residue) with the highest ACE [atomic contact energy (-183.29 kcal/mol)] and AlgM (M residue alone) gave the least ACE (-1.18 kcal/mol) with that of *C. histolyticum* collagenase.

TABLE II

Binding site analyses of Alginate oligosaccharides (Alg2-Alg6), L-cysteine and N-Acetyl-L-cysteine (NAC) with that of Clostridium histolyticum collagenase using PatchDock.

Ligand	-ACE (kcal/mol)	Interaction of amino acid residue	Bond distance (Å)
AlgM (M residue alone)	1.18	Asp418 Thr421 Arg443 Tyr525 Tyr528 Tyr533	3.29 2.47 2.07 & 2.99 3.20 2.87 3.33
Alg2 (M-G residue)	65.54	Tyr618 Asp737	3.08 3.40
Alg3 (M-G-M residue)	133.98	Arg194 Gly250 Asn251 Phe295	2.51 & 3.44 3.26 & 3.40 2.42 2.90 & 3.35
Alg4(M-G-M-G residue)	135.68	Asn210 Asn213 Gln215 Asn251 Asp296	2.13 & 2.37 3.13 3.09 2.86 3.09 & 3.49
Alg5 (M-G-M-G-M residue)	183.29	Arg194 Asn210 Gln215 Gly250 Asn251 Phe295 Asp296	2.50 3.05 & 3.06 3.10 & 3.42 2.99 2.47, 2.79, 3.12 & 3.44 3.08 2.85 & 3.07
Alg6 (M-G-M-G-M-G residue)	79.77	Pro572 His615 Tyr618 Glu619 Lys667 Asp744	3.37 3.43 1.93 & 3.42 3.39 3.24 2.94
L-Cysteine	85.93	Thr421	2.37, 2.82 & 3.9
N-Acetyl-L-Cysteine (NAC)	103.89	Thr728	3.48

This finding was in good agreement with earlier reports, where low viscosity sodium alginate (LVA) has shown to inhibit MMP 2 activity in WEHI-164 cells (21), while silver treated alginate exhibited good binding affinity towards MMP 2 (14). Apart from these, phlorotannins from *Ecklonia cava* (brown algae) and fucoidan (bioactive compound from brown algae) have shown to inhibit both MMP 2 and MMP 9 activities. Similarly, alginate rich *Fucus vesiculosus* (brown algae) has shown to inhibit *C. histolyticum* collagenase activity (4).

According to Eckhard and co-workers (22), ColG (collagenase G from *C. histolyticum*) has one activator domain (Tyr119-Asp388), one peptidase domain (containing two sub-domains Asp398-Gly670 & Asp679-Asp790), one polycystic kidney disease (PKD)-like domain (Asn795-Asn880) and two collagen binding domains (CBD). In the present study as shown in the Table II and Figure 2, none of tested ligands have shown to interact with either PKD-like domain or CBD of ColG. Interestingly, Alg2 (M-G residue) has shown to interact with Tyr618 and Asp737 amino acid residue of *C. histolyticum* collagenase, whereas ligands (namely sitostenone, erucylamide, vitamin E and xylenol) have shown to interact with PKD-like domain of ColG (23).

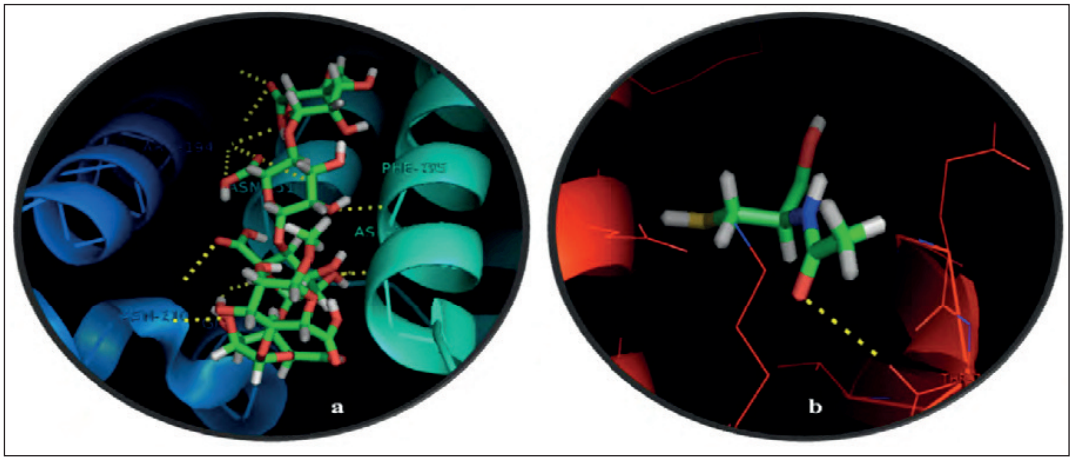

Fig. 2 *Represent (a) Alg5 (M-G-M-G-M residue) & (b) N-Acetyl-L-cysteine docked with bacterial collagenase using Pacth Dock.*

CONCLUSION

In the present study, all the tested ligands have shown to dock and bind to *Clostridium histolyticum* (bacterial) collagenase. A gradual increase in atomic contact energy (ACE) value was observed for alginate oligosaccharides from Alg2 (M-G residues) to Alg5 (M-G-M-G-M). On the other hand, N-Acetyl-L-cysteine (NAC) exhibited high ACE value when compared to L-cysteine. Thus, the obtained results have shown the potential activity of these alginate oligosaccharides (Alg2-Alg6) as bacterial collagenase inhibitory agents.

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Travelling all over China. Cultural heritages of two ancient civilities, Chinese and Italian, at the base of the progress. Part III

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It is a common responsibility of all mankind to preserve the natural and cultural wealth and heritages created by our ancestors.
Due to culture traditions and unbroken history of several thousand years of China and Italy, many historical treasures have been created and remain as precious heritages to preserve for the incoming generations. Both those Countries have played an important role influencing and guiding culture and civilization of other peoples in east and west respectively.
Italy was at the centre of the culture in Europe and part of Africa making roads, theatres and palaces, during the ancient Roman and Renaissance period, as well as China in Eastern Asia by its emperors had supreme status and priority using the entire country's labour and financial power to build imperial palaces, gardens, temples and mausoleums. Thus, the Imperial Palace (Fig.109) of the Forbidden City and the Temple of Heaven (Fig.110) in Beijing, epitomize not only the ancient Chinese architecture but also the imperial culture, as well as Coliseum (Fig.111) and Imperial forum (Fig.112) in Rome glorify the great engineering abilities and the imperial culture of the Antique Romans not only by their engineers and architects but also by philosophers, poets and writers.

Fig. 109 *A general view of the Forbidden City, Beijing.*

Fig. 110 *Temple of Heaven, Beijing.*

Fig. 111 *An unusual view of the Colosseum.*

Fig. 112 *The Imperial forum, Rome.*

The first archaeological findings of Chinese architecture, recovered near Xi'an, were made by wood between 5000 and 7000 years B.C., while near Luoyang have been found finds dated between the dynasties Xia (21st-16th century B.C.) and dynasty Shang (16th-11th century B.C.).

During this historical period people lived into simple huts (Fig.113) while the dominant classes had more complex architectural structures (Fig.114).

Subsequently the Zhou Dynasty (770-221 B.C.) conquered the kingdom, during which the first clay roof-tile (Fig. 115) appeared as technological substitution to thatching. During this period (5th century) the use of iron determined a great development of agriculture and handicraft with production of coins and conventional weaponry. However for more then 3500 years, in China house and temples have been made prevalently by wood by an architectural style, where pillars and beams had static functions, while the walls, used to fill the open surfaces, had to acts adornments only (Fig.116). Stones were used to make terraces, banisters, pagodas, bridges, roads, steps decoration (Fig.117) and defensive structures. However, the roof was generally covered by roof-tiles made by glazed ceramic with different colours (Fig.118), the yellow ones reserved for the imperial buildings only (Fig.119). The more important palaces were decorated by fantastic animals such as fishes, dragons phoenix and lions the function of which was to protect the house first of all from fire (Fig.120).

Fig. 113 *Huts houses during Shang Dynasty.*

Fig. 114 *A typical architecture during the Shang Dynasty.*

Fig. 115 *Clay roofless-tiles.*

Fig. 116 *Archaeological findings of Shang Dynasty.*

Fig. 117 *Pagodas and bridge made by stone.*

Fig. 118 *Ancient ceramic colored tiles.*

Fig. 119 *Yellow glazed tiles reserved to emperor.*

Fig. 120 *Palace roof-tiles decorations with fantastic animals.*

In conclusion, differently from the western and, of course, Roman/Italian culture the Chinese brickwork was not only so important to make house and buildings, but fundamentally to make decorated terraces, towers, and roads that resulted very important for eastern culture (Fig.121 and 122).

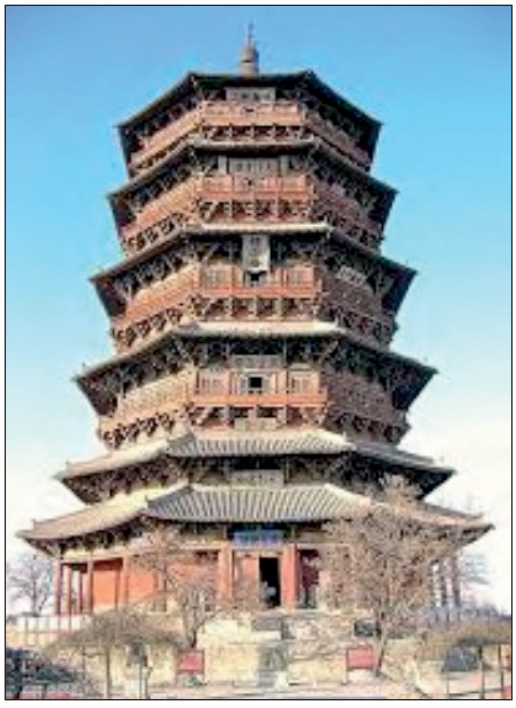

Fig. 121 *The Chinese pyramid.*

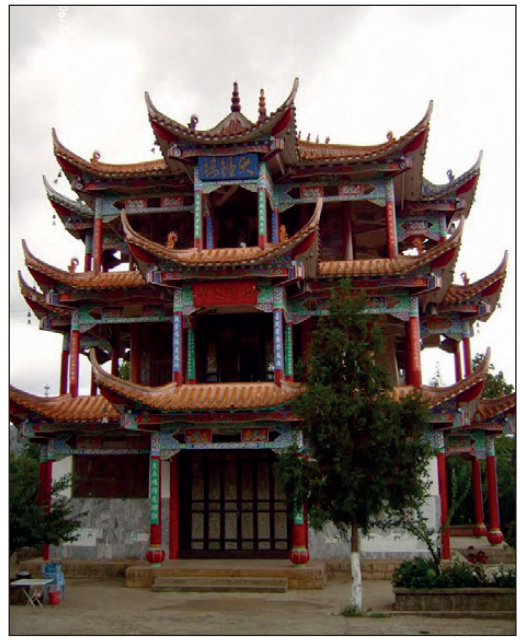

Fig. 122 *A Chinese decorate tower.*

Chinese dynasties

The first Chinese dynasty (Fig.123), of which until now no archaeological finds have been found, is considered the ***Xia*** Dynasty from 2205 to 1766 B.C. Differently from Xia, numerous written testimonials have been recovered together with archaeological finds on the ***Shang*** Dynasty (1800-1122 B.C.). The society in this period, fundamentally based on agriculture, was characterized from many wars, so that the capital was changed for six times. From 1122 to 771 B.C. the kingdom was in the hand of ***Zhou Western*** Dynasty. In this period the imperator Zhou (Fig.124) recognizing the *Sky* as superior entity, and himself as *Son* of the *Sky*, became the intermediate between God, earth and humans. From that period all the Chinese imperators had this title and Chinese people were divided in classes beginning from the more important *wen* (man of letters) to *nong* (country people), *gong*

(workman) and the last *Shang* (merchant). Moreover, the writing (Fig.125) became very important so that who was able to write arrived at the top of the social scale. Also if the ***Zhou eastern*** Dynasty (771-476 B.C.) was a period of wars in the same prosperous period was born Laozi (Lao Tzu) father of Taoism and Confucius of the Confucianism (Fig.126), giving the starting of the more famous philosophical schools of China.

After a period of many little kingdoms and wars that don't recognize the sovereignty of the imperator (476-221 B.C.), ***Qin*** Dynasty (221-206 B.C.) (Fig.127), took power by the imperator Qin Shi Huang who unified weight and misuses establishing also the same way of writing in all the Chinese territory. Under this imperator the famous Terracotta Army (Fig.128) was made, represented by a collection of terracotta sculptures. Recovered in the city of Xi'an, these wonderful sculptures are composed of more than 8000 soldiers, 130 chariots with 520 horses and 150 cavalry horses (Fig.129). It represents a funerary burial art for the emperor whose purpose was to protect himself afterlife.

What happened in the same period in ancient Rome? It is remembered as a prosperous republican period during which, ***Julius Caesar*** conquered Gallia and Britannia (58-51 B.C.) stabilizing, enlarging, and fortifying the Roman territory (Fig.130).

In China, during Qin Dynasty the Great Wall was originally conceived (Fig.131), to prevent incursions from barbarian nomads into the Chinese Empire. The best- preserved section of it was built from the 14th through all the 17th century, during the ***Ming Dynasty*** (1368-1644 A.D.) (Fig.132, 133).

Fig. 123 *Ying Zheng, the first Chinese Emperor. (Xia Dinasty)*

Fig. 124 *Lao Zhou Emperor from Zhuo Dynasty.*

Fig. 125 *The Chinese art of writing.*

Fig. 126 *Image of Confucius.*

On the other hand, the Mediterranean areas were characterized by the Greek Hellenistic kingdoms, the power of Carthage in Africa, and the ***Roman Republic*** in Italy. Following the second victorious war against Carthage, Romans became the most important power in the western Mediterranean. Thus the roman culture flourished together with a new architectural style during the Roman Republic (Fig.134) and even more under the Empire. The building was strong and well-engineered with an intelligent use of vaults and arches (Fig.135).

Fig. 127 *The Chinese territory during Qin Dinasty.*

Fig. 128 *The antique Chinese terracotta soldiers.*

Fig. 129 *The funerary burial carriage.*

Fig. 130 *Roman Empire during Julius Cesar period.*

Fig. 131 *The Great wall during Qin Dynasty.*

Fig. 132 *China territory during Ming Dynasty.*

Fig. 133 *The Great Wall during Ming Dynasty.*

Fig. 134 *Roman architecture during the republican period. Temple of Ercole Vincitore.*

Fig. 135 *Ancient Roman temple in Nimes during the empire period.*

A crucial factor for these important constructions was the invention of Roman concrete (opus caementicium) (Fig.136) that, together with a sound of knowledge of other building materials, enable Romans to achieve unprecedented success with conduction of imposing infrastructures for public use, such as amphitheatres, baths, bridges, domes and temples.

Fig. 136 *Example of a Roman reticulum bind by opus caementicium.*

Coming back to China, the **Han** family, who dominated from 206-B.C to 220 A.D., was certainly the most important and long-lasting Chinese dynasty during which the first imperator Liu Bang re-organized the agriculture giving the land to the countrymen, contemporary reducing their taxation. During this prosperous period the capital was again Chang'an (actually Xi'an) than in ancient Chinese means *perpetual peace* (Fig. 137). It was the largest city with about 1 million of habitants, just as the Ancient Rome in the empire period!

Fig. 137 *A ceremony in the antique Xi'an (left) and a building (right).*

Under the empire of Wen Di, successor of Liu Bang, the first university of the world was founded and the first *mandarins* were graduated, helping the emperor and the succeeding imperators to govern China for about 2000 years. In this period the paper was also invented (Fig.138) and Buddhism entered in China for the first time.

Fig. 138 *Making paper on an ancient Chinese paintings.*

What about the Ancient Rome in this period? It was the golden period of the Roman western empire, which started with ***Augustus*** (27 B.C. - 14 A.D.), continued with Tiberius (14-37 A.D.), Caligula (37-41A.D.), Claudio (41-54 A.D.),

Nerone (54-68 A.D.), Vespasian (69-79 A.D.), Traiano (98-117 A.D.), Adriano (117-138 A.D.), Marco Aurelio (161-180 A.D.) and Caracalla (211-217), only to remember the most famous imperators (Fig.139-142). During this period Rome, as capital of a vast empire was enriched of marmoreal temples with ample colonnades, thermal baths, theatre and forum where people discussed with politicians, writers and philosophers about the State organization and its wellbeing.

Fig. 139 *The Imperator Augustus.*

Fig. 140 *The Imperator Marco Aurelio.*

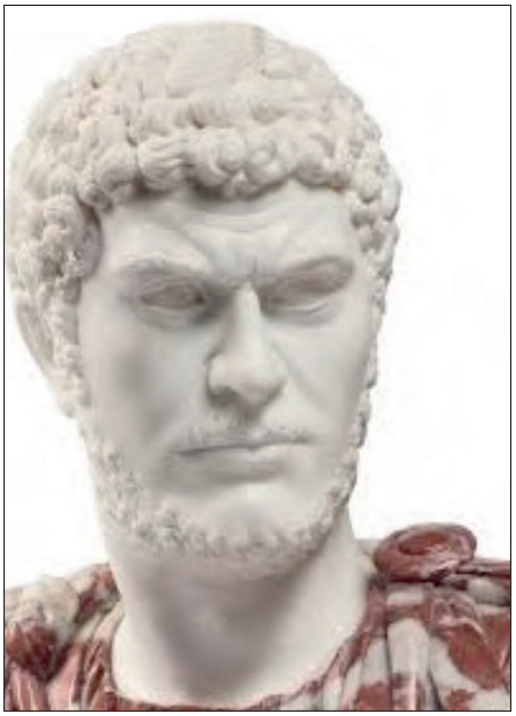

Fig. 141 *The Imperator Caracalla.*

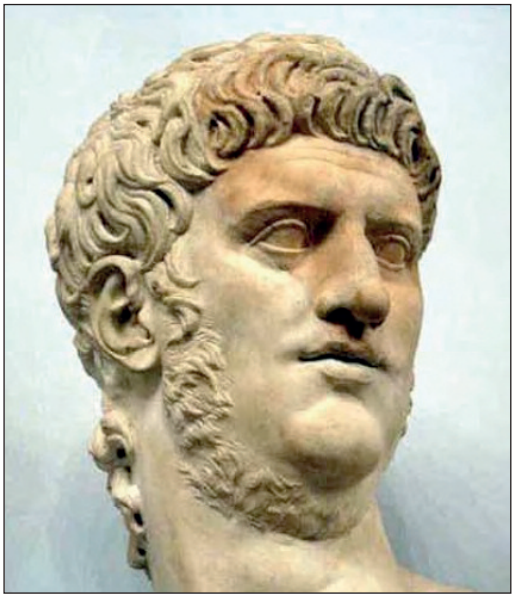

Fig. 142 *The Imperator Nerone.*

On the other hand, the Chinese empire arrived to have the largest territory (Fig.143) during the ***Yuan*** Mongolian Dynasty (1276-1368 A.D.) with Genghis Khan and Qubilay Khan. During this period the major roads were made to ameliorate the internal and external commercial and political interchanges, more than 42,000 new monasteries were built, the Dalai Lama as spiritual leader of Tibetans was officially recognized, while the first intellectual and commercial interchanges between eastern and western cultures were established, thanks also to travellers as Marco Polo.

Fig. 143 *The Genghis Khan period. Territory of China.*

During this period in Italy was born the poet Dante Alighieri (1265 A.D.), famous for having written the Divine Comedy (Divina Commedia) long narrative poem divided into three parts Inferno, Purgatorio and Paradiso (Hell, Purgatory, and Heaven). It is considered a preeminent literary work that has influenced not only the Italian but also the European literature. Dante together with Boccaccio have given the first impulse towards a unified, standard Italian language. In the same period the famous painter, architect and sculptor Giotto (1266-1336 A.D.) was also living and working. Music has always played an important role in Italian and European culture. Many music instruments and music terms in the western culture (Fig.144), in fact, were, invented in Italy and many of the existing classical music forms such as symphony, concerto and sonata, can trace their roots back to innovation of sixteenth and seventeenth century. The most famous renaissance composes include Alessandro Scarlatti, Corelli and Vivaldi of the baroque period, Paganini and Rossini of the classical period, and Verdi and Puccini of the romantic ones.

Fig. 144 *Instruments of Western culture.*

After Mongolian arrived in China the ***Ming Dynasty*** (1368-1644 A.D.) who edified the *Forbidden City* and the wonderfully *Temple of Heaven*, restructuring all the *Great Wall* also. Moreover during this period were re-stamped many classical, agricultural and architectural books with an encyclopaedia composed of 22,937 books. During the same period the famous explorer Zhen He (1371-1434 A.D.) arrived with a merchant fleet in India and Persian Gulf. Always during the Ming Dynasty, it is to remember the Italian Matteo Ricci (Li Madou) (1574-1610 A.D.) (Fig.145) who had an interesting impact on Chinese culture. This Jesuit missionary, encountering a Chinese society with high moral values, was of the opinion that the ethical and social doctrine of

Confucianism could be complemented with the metaphysical idea of Christianity. Moreover, Christianity shared elements with Buddhism and Confucianism such as belief in afterlife, with the idea of heaven and hell and the practice of celibacy.

Fig. 145 *Matteo Ricci (Li Madau), Jesuit in China.*

Li Madou was a pioneer who, disseminating the western knowledge about mathematics, astronomy and geometry, and distinguishing religion from culture, recognized the possibility for people to become Christians without the necessity to adopt the European culture at the same time. He was also the first European to reach China presenting the music instrument Harpsichord (Fig.146) to the Ming imperial court, training four eunuchs to play it.

At this purpose, it is to remember that in ancient China the position of musicians was much lower than the painters, though music was seen as central to the harmony and longevity state (Fig.147). Thus, every emperor took folk songs seriously sending officers to collect the popular will in all the empire territory.

Fig. 146 *The musical instrument Harpsichord.*

Fig. 147 *The musical instruments in ancient China.*

On the other hand in the same period in Italy, famous painters such as Piero della Francesca (1416-1492 A.D.), Sandro Botticelli (1445-1510 A.D.) and Leonardo da Vinci, painter, sculptor, inventor and architect were living and working

in Italy (1452-1519A.D.), together with Michelangelo Buonarroti (1475-1564 A.D.) sculptor and architect, Michelangelo Merisi known as Caravaggio (1571-1610 A.D.) painter, Gian Lorenzo Bernini (1598-1680) sculptor/architect and Cristoforo Colombo (1492 A.D.) the known navigator who discovered the America.

The last Chinese imperators have been represented from the ***Qing Dynasty*** (1644-1911 A.D.) before the ***Popular Republic of China*** (1912 A.D. until today). In the same period Italy was unified as Country (1861 A.D.), Antonio Canova (1757-1822 A.D.) became famous for his neoclassical sculptures and Guglielmo Marconi (1895 A.D.) invented the telegraph.

GROTTOES

China

Grottoes are Buddhist architecture that, originated from India, served to practice religion. The more known grottoes, decorated with painted sculptures, were built along the Yellow River in North China. Among the more famous it is necessary to remember 1) the Mogao Grottoes located on Mount Mingsha in Dunhuang Gansu Province; 2) Dazu Rock Carvings; 3) Longmen Grottoes, and 4) Yungang Grottoes, started and had its pick in the Tang Dynasty.

1. The Duhuang Mogao Grottoes (Fig.148), are known as the largest existing Buddhist artistic thesaurus in the works. They are represented of 735 grottos, 492 of which have coloured statues and fresco (Fig.149).
2. The creation of the Dazu Rock Carvings (Fig.150-152), located in Chongqing, started in the Tang Dynasty (618-907 A.D.), having its pick in Song Dynasty (960-1279 A.D.), ending in the Ming (1368-1644 A.D.) and Qing dynasties (1644-1911 A.D.).

Fig. 148 *Buddha figures in Mogao grottos.*

Fig. 149 *Paintings in Mogao grottos.*

3. The Longmen Grottoes (Fig.153, 154), located in Laoyang Henan Province, is one of the three Ancient Chinese Buddhist Grottos Art thesaurus. As an embodiment of imperial will and behaviour, these grottos have strong features of state religion.

Fig. 150 *Representation of devils (Dazu Rock).*

Fig. 151 *Dazu rock carvings.*

Fig. 152 *Dazu rock carvings.*

Fig. 153-154 *Longmen grottos.*

4. The Yungang Grottoes (Fig.155), located in Datong, Shanxi Province, represent an extraordinary art of the 5th-6th centuries, when Buddhism was first introduced into China. They are represented from 252 grottos, holding 51,000 statues.

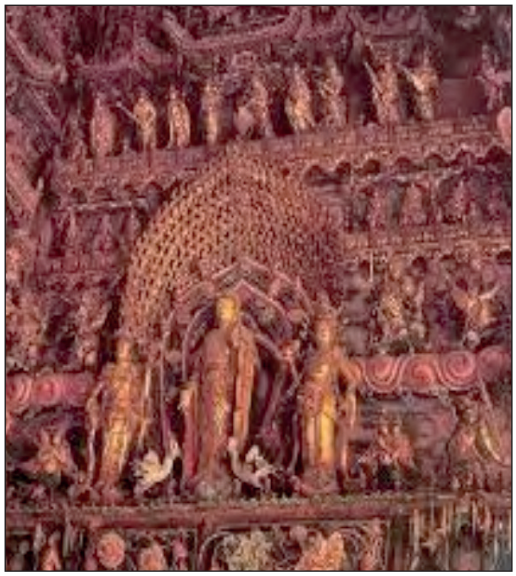

Fig. 155 *Yungang grottos.*

Italy

The Italian Renaissance garden, as reported previously, was a new style of garden emerged in the late 15th century in villas in Florence Rome and other Italian locations. These gardens, enriched with grottos and statues, differently from China, had only a cultural and not religious significance. Among the more known:

1. Boboli gardens in the Pitti Palace have the largest green area in Florence, commissioned in 1549 A.D., was residence of Medici family also (Fig.156).
2. Bomarzo monster in Viterbo, near Rome (Fig.157).
3. Buontalenti grotto made by the famous painter and architect Giorgio Vasari (1511-1574 A.D.), Florence (Fig.158).
4. The Giant sculpture by Gianbologna (1529-1608 A.D), famous Italian sculptor, is located in villa Deminoff, Florence (Fig.159).

Fig. 156 *Boboli Gardens, Florence, Italy.*

Fig. 157 *Monster in Bomarzo Garden, Viterbo, central Italy.*

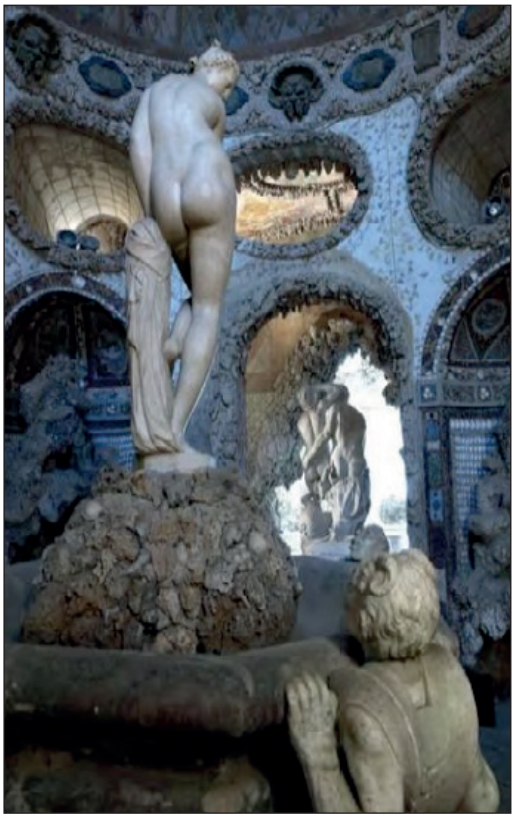

Fig. 158 *Buontalenti grotto by Giorgio Vasari, Florence.*

Fig. 159 *The Giant sculpture by Gianbologna located in villa Deminoff, Florence, Italy.*

PAINTINGS

China

Traditionally Chinese paintings were done not only on rice paper or thin silk, but also on walls, porcelain, and glass and lacquer ware. The first paintings were recovered from the Han Dynasty (202 BC) to Tang Dynasty (618-906 A.D.) by artists working at the emperor court. However, the time from the Five Dynasties period (220 B.C. to 960 A.D.) to the northern Song Period (960-1279 A.D.), known as the Golden Age of Chinese Landscape Painters, outlined mountains and Myths, while during the Yuan Dynasty (1279-1368 A.D.) poetry and calligraphy was started to be added to their works, for expressing their thoughts and feelings. The colour printing techniques were perfected during the Ming Dynasty (1368-1644 A.D.), while the western influence arrived in the last 1800-1909 A.D. period of Qing Dynasty. Some examples of the more known paintings of the different historical periods are reported just to compare the two different cultures: Cinese and Roman\Italian.

Han Dynasty (206 B.C.-220 A.D.)

Fig. 160 *Painting of Han Dynasty.*

Fig. 161 *Painting on a tomb of western Han Dynasty.*

Northern and Southern Song Dynasty (420-581 A.D.)

Fig. 162 *In presence of the Imperator.*

Fig. 163 *Working at home.*

Fig. 164 *Flowers and animals.*

Yuan Dynasty (1279-1368 A.D.)

Fig. 165 *A lady offers tea.*

Fig. 166 *Painting on silk.*

Fig. 167 *The Painter.*

Ming Dynasty (1368-1644 A.D.)

Fig. 168 *At the court of the Imperator.*

Fig. 169 *Painting with perspective.*

Fig. 170 *The writer.*

Qing Dynasty (1644-1911 A.D.)

Fig. 171 *Painting on glass.*

Fig. 172 *Painting on glass.*

Fig. 173 *The lady at home.*

Italy

All the art of the early Roman republic period (500-200 B.C.) was influenced by the Greek art of Magna Grecia (Southern Italy) and by Etruscans (Central Italy). Roman art in this period included architecture, paintings, sculptures, and mosaics made of colored little stones used to make figures and environments. Unfortunately of the vast body of Roman paintings only few archaeological finds remain, recovered especially in the cities of Pompei and Herculaneum covered by the land for centuries after the Vesuvius eruption in 79 A.D. However, from 200 to 60 B.C. in Roman wall painting the style of *incrustation* was used.

It consists of the wall containing colour patches of blocks resembling the marble walls by a variety of predominant colours such as yellow, purple and pink. The paintings that report illusions of imaginary scenes, wanted to give off the illusion to look through a window, just as the Chinese were doing by their gardens.

During the period 27 B.C.-14 A.D. Rome prospered during different imperators achieving administrative efficiency by centralizing government taking control of the treasury and expanding the civil service with vast programs of public works, including roads, new aqueducts and canals. In this period the provinces Britannia, Mauritania, Thrace, Lycia and Phamphylia were added to the empire, the expansion of which begun with Julius Caesar for continuing with the famous imperators Augustus, Tiberius, Claudio and Nerone. Ancient Romans was first of all engineers and architect capable to make wonderful constructions. However, many houses and public libraries and thermal baths were enriched by paintings and sculptures also.

Differently from China, where the Han ancestral culture dominated continually until 1276 A.D. with the entering of Mongolians, in Italy from 500 to1000 A.D. successive waves of conquerors (Byzantines, Longobards, Franks and Arabs) captured and recaptured strategic cities its peninsula and Sicily. Thus the art has been influenced by many different styles from Ostrogoth (493-552 A.D.), Byzantine (476-1071 A.D.) and Longobard (568-774 A.D.) kingdoms to Arab (827-1061 AD) and Norman (1061-1091 AD) dominations. The so called Middle age (1100-1300 A.D.) began during the Byzantine empire and was weakening under the pressure of Arabs and Longobards with the invasion of

Charlemagne in 773, who established the kingdom in Italy and the Papal States. This was a period of wars during which merchants and lord fought for the control of towns with the help of Pope and the emperor. Italy was divided in many feuding city-states and every city-state was divided between their supporters, i.e. Pope or Emperor. Thus began a political development with the transformation of medieval communities into powerful city-states, modeled on ancient Roman Republicanism. The republic of Venice, Florence, Genoa and Pisa, among others rose to great political power and paved the way for the Italian Renaissance (i.e Rebirth period) with the resurgence of western civilization. This particular period of the history (1400-1600 A.D.) was characterized by a surge of interest in classical scholarship and values, with a growth of commerce and inventions and revival of science and classical learning. People was encouraged to use experimentations and observations to solve earth problems. Thus, for example, Leonardo da Vinci created scientific reproductions of objects ranging from flying machines to submarines, promoting studies on human anatomy also.

The projects of urban renewal and expansion initiated in Rome with the Renaissance, reach a spectacular apex during the 1600 by artists, coming from elsewhere in Europe, to visit Rome for studying the masterpieces of antiquity. They executed commissions for the Popes and a secular rich clientele, so that the Baroque style took form, shaped by the hands of great masters such as Gian Lorenzo Bernini (1598-1680 A.D.), Francesco Borromini (1599-1667 A.D.) and other sculptors and painters reported below.

Archeologists describe the development of Roman painting in four styles. The First Style (about 200-60 B.C.) was characterized from a simulation marble of various colours with Hellenistic influence. The decline of the First Style coincided with the Roman colonization of Pompei (80 BC). In the Second Style, fresco artists imitated architectural firms by pictorial means (as in Villa Fannius Synistor of Boscoreale). Under Emperor Augustus in the second half of the first century B.C. there was impulse to innovate with the advent of the Third Style (from 20 B.C. to 20 A.D.), evidenced in Boscoreale villa, in Pompei and Ercolano. Warm colours were used to advantage in the depiction of scenes drawn from mythology. The Fourth Style revived large-scale narrative painting and panoramic vistas.

Together with paintings mosaics also, dominated from the Greek influence, had a great success in the ancient Roma. The dominant Roman style in Italy was the use oh black and white tesserae used to represent marine motifs, especially to make floor bath, as in Caracalla Thermae. However many coloured mosaics and paintings were used to decorate Roman villae, as recovered in Pompei, Ercolano in Campania and Piazza Armerina in Sicily.

Pre and Past Augustus period (40 B.C.-220 A.D.)

Fig. 174 *Roman Mosaique.*

Fig. 175 *Painting at Villa di Boscoreale (50-40 B.C.).*

Fig. 176 *Roman personalities.*

Fig. 177 *Painting in Pompei.*

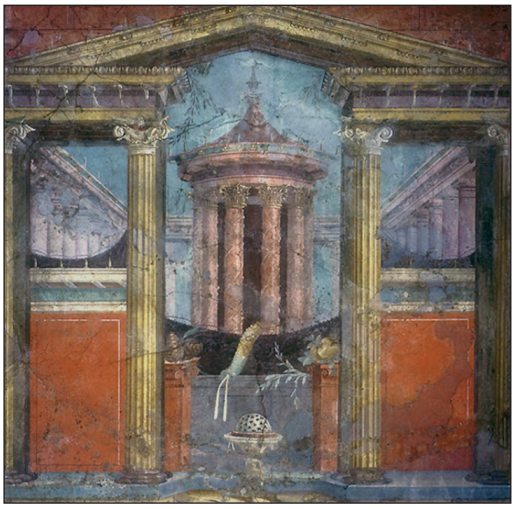

Fig. 178 *Cubiculum Villa P. Fannius at Boscoreale.*

Fig. 179 *Mosaic Villa Armerina.*

From Ostrogoth to Arab/Norman period *(493-1061 A.D.)*

Fig. 180 *Theodora mosaic, Basilica of San Vitale, Ravenna.*

Fig. 181 *Mosaic, Basilica of San Vitale, Ravenna.*

Fig. 182 *Mosaic, Duomo of Monreale, Sicily.*

Fig. 183 *Moisaic of Secrets, Otranto, Puglia.*

Medioeval period (1278-1368)

Fig. 184 *Cimabue, Maestà in Santa Maria dei servi.*

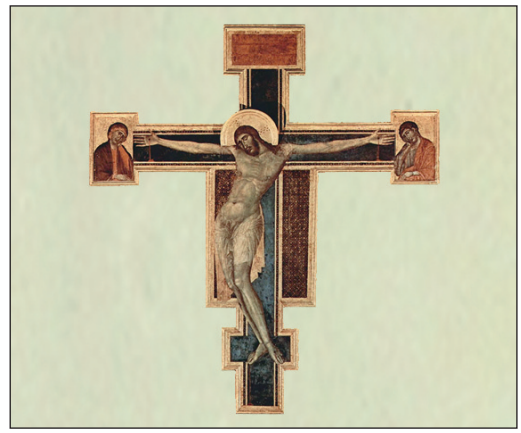

Fig. 185 *Cimabue cross in Santa Croce.*

Fig. 186 *The Annunciation, Giotto.*

Fig. 187 *Giotto, Cappella degli Scrovegni.*

Renaissance period (1368-1644)

Fig. 188 *Dukes of Urbino, Piero Della Francesca (1416-1492).*

Fig. 189 *Madonna with baby, Raffaello Sanzio (1483-1520).*

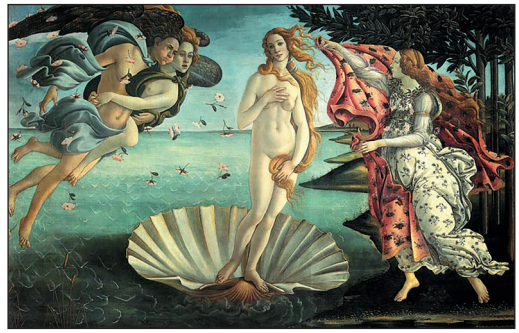

Fig. 190 *Venus birth, Sandro Botticelli (1445-1510).*

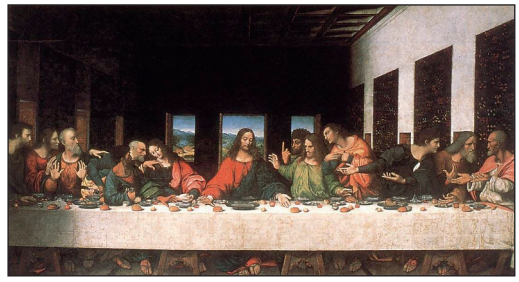

Fig. 191 *The last Dinner, Leonardo da Vinci (1452-1519).*

Fig. 192 *Sistine Chapel by Michelangelo.*

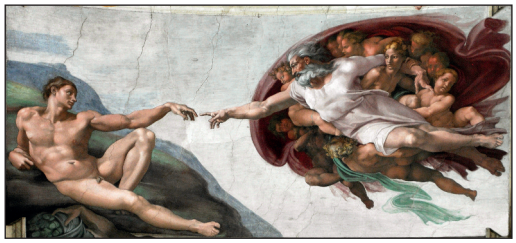

Fig. 192 bis *Sistine Chapel, Michelangelo Buonarroti (1475-1564) (particular).*

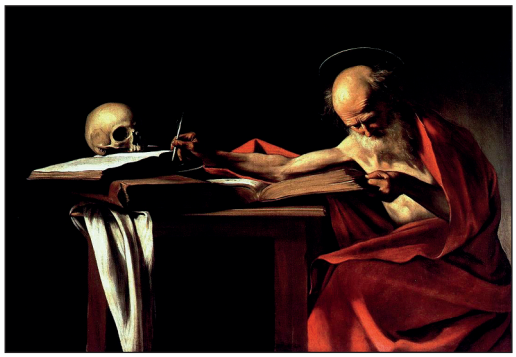

Fig. 193 *San Girolamo, Michelangelo Merisi called il Caravaggio (1571-1610).*

Baroque and Modern period *(1764-1911)*

Fig. 194 *Sibilla Persica, Giovanni Francesco Barbieri called il Guercino (1591-1666).*

Fig. 195 *Madonna with baby, Giovanni Francesco Barbieri called il Guercino (1591-1666).*

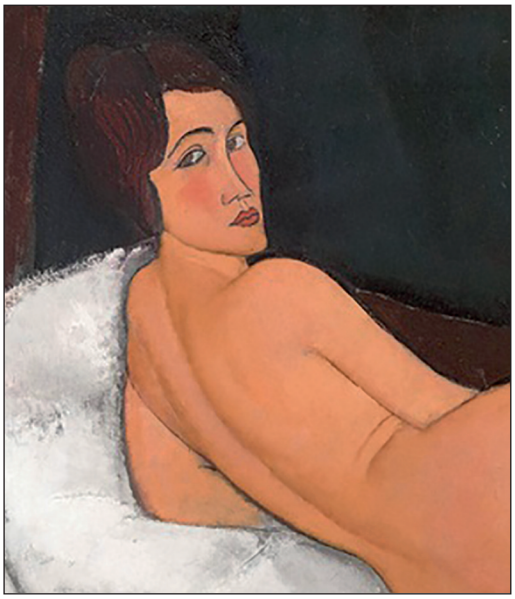

Fig. 196 *Nude woman, Amedeo Modigliani (1884-1920).*

Fig. 197 *Redheaded woman, Amedeo Modigliani (1884-1920).*

to be continued ...

Applied Nanotechnology

By VI Kodolov, GE Zaikov, AK Haghi

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Nanotechnology is the process by which components, processed and manipulated at the nanoscale comprising from 1 to 100 nanometers, try to mimic biological structures. It is a highly promising field, where disciplines such as chemistry, physics, biology, engineering, and medicine interact with nanostructured materials offering potential solutions in multiply practical applications, applied first of all in theranostic (diagnostic and therapeutical applications) drug delivery, and tissue engineering but the use of synthetic and natural biomaterials.

This book, organized in **4 Parts** and **20 chapters**, reports and discuss the advanced nanostructured materials and tools, characterizing and predicting their properties and behaviour. In particular the authors, coming from industry and academia, cover various applications of biomaterials, investigating their structure and properties reported and discussed in occasion of the Fifth international Conference: *From Nanostructure, Nanomaterials and Nanotechnologies to Nanoindustry* held in April 2015 at Kalashikov Izhevsk State Technical University, Russia.

Part I: *Nanostructures and Nanosystems for Improvement of Materials and Objects*, comprising 4 chapters, reports on **chapter 1** a mobility-based post-synthetic route useful to optimizing the surface-enhanced Raman scattering(SERS)-active nanostructures by the use of metal Nanoparticles (NPs).

Basically this Nanosystem, ideal in a wide range of practical applications, consists on antibody-functionalizing silver-NPs held together by a Raman reporter and stabilized by the presence of polyethylene glycol chains as capping agent. The sensitivity, the robustness and the high reproducibility of the successfully engineered SERS bioassay based on these active nano-assemblies seems to provide a suitable platform for developing multiplexed sensitive detection of proteins and other target molecules, not possible with the colorimetric and fluorine trip ELISA analogs. On **chapters 2-4**, are reported many topics such as the problem regarding the redox synthesis of metal/carbon nanocomposites in nanoteactors of polymeric matrices.

The new possibilities to develop new ideas on the base of mesoscopic physics principles for the self-organization synthesis of polymeric matrices are discussed. Thus, it has been shown that the energetic characteristics of Nanocomposites result more important for their activity determination in comparison with their size. Their vibration energy depends, in fact, on their average masses. Many other short communications discussed the properties of different nanostructures and nanosystems based on the use of tetrapyrrolic photosensitizers, phthalocyanines for the development of nano sensors, nano aggregates for early diagnosis, or the photodynamic therapy, reporting interesting and practical results.

Part II: *Modeling in Sphere of Nanoscience and Nanotechnology* describes by **chapters 5-8** the geometry and the entropic spatial-energy interactions of the micro-nanoparticles, reporting also a proposed method to determine their elastic modulus and modelling processes of solid or layered nanostructures. Main components of organic compounds, constituting 98% of cell elemental composition are carbon, oxygen, hydrogen and nitrogen.

Carbon is the main conformation center of different structural ensembles, including the formation of cluster compounds. Clusters are aggregates of anything from a few to many atoms or molecules. Therefore, analyzing the mechanism of formation of carbon clusters it is possible, by analogy, to understand the geometry of the hexagonal cell structures and the interactions between atoms, ions or molecules expressed by a variety of potential energy functions.Thus,the concept of the entropy of spatial-energy interactions ,appear based on the second law of thermodynamics on the static energy, with rules independent of microscopic models. This concept is reported and discussed together with the variability of entropy in biochemical processes, economics, and engineering systems.

Part III is entirely dedicated to the *Investigation of structure and Properties of Nanostructures, Nanosystems, and Nanostructured materials* by **chapters 9-11**. Thus, it is reported a review on the development of the X-ray photoelectron magnetic spectrometers and their use to investigate the transformations of substances participated with nanostructures by electro structures.

The results of X-ray photoelectron spectroscopy investigations of different processes as well as the development of unique X-ray photoelectron magnetic spectrometers are, therefore, discussed.

Moreover, the influence of minute additional of carbon metal-containing nanoforms is studied on the polymer structure for polycarbonate, polymetylmethacrylate and polyvinyl alcohol, compounds which have different content of oxygen. Thus, it has been shown that, the temperature growth blocks the development of the nanostructure self-organization in the medium; the degree of nanostructure interaction with a polymer is determined by the content on nanostructures and their activity in the medium, and the change of the polymer structure is accompanied by the change of its technological properties.

In conclusion the larger is the content of oxygen atoms bound to carbon atoms in the polymers, the larger is the degree of the polymer modification and the smaller is the percentage of nanoforms required for the beginning of modification. However, for the preparation, validation and application of nanoscale and nanostructured metal powders and their multi component systems, required from modern material science, it is necessary the investigation of their composition and structure. Thus the need to use the entire methods of X-ray diffraction together with spectroscopic and other physical methods, all reported in this part of the book.

Part IV *Production of Nanostructured Materials and Investigations of their Properties* is described in **chapters 12-16.** Among the productive processes, the electrospinning technology has gained much attention mainly because considered the most cheap and simple method to produce non-woven tissues with nanoscale diameters to be used in medicine. The most significant challenge in this process is to obtain uniform, consistent, and reproducible nanofibers and non-woven tissues, having high surface and high porosity to make scaffolds structured as the native extra cellular matrix (ECM).

Compared to other scaffolding materials, electrospinning allows creating nanofibers from organic or inorganic materials of high Young's modulus and, therefore, of an higher mechanical strength. These scaffolds, being prevalently used to make engineered tissues, have to be manufactured with appro-

priate and functional biomaterials for optimum, robust, long-life performance when implanted in the human body. However, the processing conditions play a major character in the fiber formation during the electrospinning technique, being governed by the external electric field produced by the applied voltage caused by charges on the jet surface. The viscosity, polymer concentration surface tension, and polymer molecular weight are also important for the morphology of the electrospun fiber, being linked to one another. By increasing the molecular weight smooth fiber will be obtained with a larger average diameter.

On one hand, a lower surface tension of the spinning solution, for addition of a surfactant, helps electrospinning to occur at a lower electric field. On the other hand, an increase of conductivity, obtained by the addition of ionic salts, favours forming of thinner fibers as well as an increase in the applied voltage.

However it is to underline that the high surface area of electrospun nanofibers has shown to be efficient for fluid absorption and dermal delivery.

Thus, by the electrospinning technique it is possible to produce effective scaffolds that permit the oxygen permeation while protect wound from infection and dehydration maintaining the necessary level of waters, contemporary impeding the proliferation of pathogenic microorganisms. Moreover, electrospun nanofibers may be used to make a controlled release system, loading active ingredients into their structure to obtain their release at a predictable rate and time. The high specific surface area and short diffusion passage length, in fact, give the nanofiber drug system higher overall release rate than the bulk material. The relative release can be finely controlled by modulating nanofiber morphology, porosity, and composition. However, as the drug delivery in the form of nanofibers is still in the early stage exploration, a real delivery mode after production and efficiency has yet to be determined in the next future. Apart the use of nanofibers as medical devices, they can play an important role in textile applications as protective clothing and other functional fabric materials. Nanofibrous membranes, in fact, are capable of neutralizing chemical agents and trapping aerosol particles without reducing the clothing' air and water vapour permeability, because of their high specific surface area and high porosity but small pore size compared with conventional textiles.

These an other news are reported on **Part V** *Applications of Nanostructured Materials and Nanotechnologies* by **chapters 17-20**.

This interesting book, written from well know scientists, reports the last developments and trends in the field of nanotechnological materials and applications that had a major commercial impact in these last years, during which the integration of clinical and engineering approaches became more stringent. The description of the nanostructures and nanosystems properties, the nanoscale technologies together with the last methodologies used for the nanomaterials control, and the combining discussion of the clinical aspects with the material science and engineering perspectives, may be of great utility for chemists, medical doctors, chemical, engineers, technologists of both the chemical and medical community with academic or industrial background, that are interested to deepen the knowledge on the nanotechnology of advanced nanomaterials.

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Nanobiomaterials. Developmant and Applications

by DK. Yi and GC. Papaefthymiou

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Nowadays nanobiotechnology and nanobiomaterials have become the most interested topics of innovative process and raw materials, respectively interesting research and development of materials characterized for their particularity and unique physicochemical and biological properties. Nanobiotechnology, in fact, describes and represents the convergence of molecular biology and engineering, studying how biological machines could work and biological motifs could be adapted to improve the existing nanotechnology.

This the fundamental aim of the book that, as part of *Advances in Materials Science* and *Engineering*, is organized in four parts and 12 chapters.

Part I - *Nanomaterials in Nanobiotechnologies: Preparation, Characterization, and Applications*, is composed by **7 chapters**. Materials science, in fact, draws inspiration from biology and strives to reproduce or mimic biological processes in the synthesis of materials thus; nanotechnology deals with the manipulation of matter at the atomic and molecular levels for the bottom-up synthesis of materials.

Chapter 1 *Bioinspired Magnetic Nanoparticles* reviews the biominralization of iron in biological, processes trying to explain how nature integrates hard inorganic materials with the soft organic world of biology. Living organisms, in fact, have the capability to provide chemical environments that control the nucleation and growth of unique mineral with the phases forming nanocomposite materials, such as bone or seashell. Bone, consists of hard apatite represented by the inorganic calcium phosphate dispersed within the organic collagen fiber matrix. It is interesting to remember that the mechanical properties of collagen depends from the particular regular arrangement of these materials. This natural polymer does not act as glue, but it is organized as a net in which the apatite particles are trapped and allowed to move against each other.

The bio-inspired routes of iron-based magnetic nanoparticle formation is reported and discussed in this chapter, where the biomimetic synthesis of iron oxide and ferrite magnetic nanoparticles in protein cages and viral capsids are specifically explored. The type and thickness of the encapsulating coat and how polymers and inorganic minerals, can affect the magnetic properties of the particle assembly, in fact, could be utilized to tailor the magnetic properties to specific applications.

Nanoparticles for Bioimaging is the topic reported on **chapter 2**. This technique is particularly important in cancer detection and diagnosis to provide more accurate and reliable information regarding the disease status and progression during, for example, the chemotherapy. When the drug is not

well localized in the target disease site, in fact, there is risks of side effects, because some drugs can change their biological metabolism when in an healthy tissue. It is, therefore, important the efficient and accurate delivery of drugs to the target site, obtainable by advances in Nanoscience and Biotechnology.

Thus, many polymers in nature used as nanoparticle building blocks of living systems, may be of great utility to carry small molecules. Among them polysaccharides, such as chitosan, hyaluronic acid, and dextran have shown promising potential for drug delivery in biomedical applications. This is the topic amply focused on **chapter 4** - *Polymeric Nanoparticles in Cancer Therapy*, while **chapter 3** - *Biomedical Applications of Dendrimer Porphyrin or Fhthalocyanine* has been dedicated to describe all the properties of special compounds such as dendrimers.

During the past decade, in fact, a great number of dendrimer structures have been developed and investigated owing to their structural merit and fascinating aspect. They have more binding capacity to immobilize biomolecules than conventional linear linkers due to their numerous functional groups, resulting in higher sensitivity and lower detection limits in biosensing applications. Among them, ionic poly (benzyl ether) dendrimers, having a functional porphyrin or phthalocyanine core have been intensively investigated because of their particular biomedical applications. Carbon nanotubes (CNTs) are seamlessly folded-up graphene sheets. Graphene is a one-atom-thick planar sheet of sp^2-bonded carbon atoms, while graphite consists of many graphene sheets stacked up through van der Waals interactions with an inter planar spacing of 0.335 nm. Graphene sheets may be folded in three ways generating three different tubes named armchair, zigzag, and chiral SWNTs.

The nanotubes can be dispersed in various non polar and polar solvents including water to be functionalized and used as targeted delivery of potent drug molecules such as amphoteric compounds. The high surface area of nanotubes, in fact, with controllable density of functionality, translated into the materials proven to be highly unique and very efficient drug delivery vehicles. However, since raw or purified unfunctionalized CNTs are potentially toxic to humans, strict industrial hygiene measures should be taken to limit exposure during their manipulation. Structure, functionalization, toxicity, pharmacokinetics and the CNTs uses are reported on **chapter 5** - *Carbon Nanotube Bioconjugates*.

The enzyme immobilization by the use of nanomaterials, nanoparticle and nanoporous supports is the subject focused on **chapter 6**. Enzymes endowed by nature are ubiquitous and versatile as catalysts that span some nanometres in size. Their activity and specificity under mild and physiological friendly conditions, allow them to be applicable to various fields, including synthesis of chemicals and pharmaceuticals, integration to biosensors, biofuel cells and many others. Unfortunately, they are sensitive, unstable, and incompatible in most cases with organic media, but can be immobilized improving and controlling their solubility, stability and catalytic reactions. Naturally, the immobilization support should be chemically and mechanically benign and stable in their repeated usage. The enzyme immobilization, in fact, has to include a facile recovery of the catalytic system from the reaction mixture at the end of the reactions so that the recovered exorbitant enzymes can be reused in the next run.

At this purpose, both physical and chemical characteristics are important in selecting the support materials in conjugation with those enzymes to be immobilized. Thus, recently polymeric materials have been used as selected support for enzyme immobilization, in combination with inorganic nanomaterials to make novel nanocomposites, as well as the single-enzyme nanoparticle has emerged as

an innovative way for its stabilization.
Moreover; nanostructured inorganic nanomaterials have shown to possess many advantages for biosensors, including lower reagent and energy consumption, minimized sample volume, less space requirement, and faster reaction kinetics.
In **chapter 7** - *Magnetically Induced Hyperthermia for Biomedical Applications* - the fundamental properties and relation mechanisms of magnetic nanoparticles are considered in connection with the efficiency of the associated magnetic fluids in magnetically induced hyperthermia. Understanding the interplay between the structural, magnetic, and colloidal parameters in a colloidal assembly of magnetic nanoparticles is, in fact, an important challenge in the field of magnetic hyperthermia. Particular emphasis is given in the dependences of the power dissipation on some parameters characterizing the magnetic fluids such as the initial susceptibility and the diameter of the magnetic nanoparticles.
In particular, it has been shown that high surface charges on the surface of magnetic nanopoarticles may prohibit nanoparticle agglomeration, keep the colloidal stability, and ensure constant magnetic heating during magnetic hyperthermia applications for several minutes at high temperatures.
Chapter 8 - *Soft Block Nanobuilding: New Preparation Routes of Sift Nanomaterials Using Biomolecules*, is the unique chapter of **Part II**.
As previously reported, biomimetics is the emerging field, of nanobiotechnology that adopts problem-solving methods inspired by nature's functions and structures, making these structures from the molecular to atomic level.
In nature, protein are rich in information that is encoded with sequences of aminoacids. Sequences are the arrangement of amino acids that determine the secondary structures of protein. Then, further folding includes the 3D architecture, directly related to functions. This phenomenon is the best example of how the encoded information can translate into performance of the nanomaterial. Small peptide fragments among the full sequence of proteins play a critical and independent role in assembly and folding, so that many investigations have been focused to identify the motif driving the peptides assembly. By controlling precisely the peptide sequences, their morphology can be tuned and functionalities can be provided selectively. Being this approach still challenging, biological molecules have been used as a template.
Biotemplating is, therefore, a technique by which it is possible to produce material with controlled morphologies and structures by employing biomaterials and templates for material synthesis. This nanomaterial synthesis refers to the efforts in extending nature-based biological templating systems to the technologically important functional materials that gave not yet been explored by nature. Thus, exploiting viruses for the fabrication of bio-hybrid materials is based on the use of highly ordered crystalline templates from which to make date and assemble materials. The functional usefulness of viruses as templating scaffolds lies in their inherent morphological features, programmable coat proteins, and possible mass production. At this purpose, the structure and dimensions of viruses are precise and uniform and the genetic and chemical modifications of their constituent units are easily attainable using basic molecular biology and chemistry. Moreover mass production is possible by proliferation in the respective hosts, which is a requirement for use in engineering applications.
In conclusion the uniform size and homogeneous morphology make viruses useful as a biotemplate to grow various kinds of materials, providing new opportunities in the understanding of biology and nanomaterials and paving the way toward bio-based hybrid nanomaterials.

Part III - *Nanomaterials and Bio-MENS: Nano-and Microscale Hybridizatin of Materials and Applications* - has composed of **chapter 9** - *Microfluidic-Based Polymer Scaffold Design for Tissue Engineering* and **chapter 10** - *Fabrication of Mobile Hybrid Microswimmers Using Micro/Nanoparticles and Bacterial Flagella*.

Micro-electro-mechanical systems, or Bio-MEMS, is a technology that in its most general form can be defined as miniaturized electro-mechanical devices and mechanical structures that are implemented using the technique of bio-micro-fabrication. Their physical dimensions can vary from over one millimeter on the higher end of the dimensional scale down to below one micron, varying from simple structures to complex and sophisticated electromechanical biological systems. However, at the frontier of drug delivery-related research and tissue engineering, engineers and biologists are working to combine robotics and control principles with microbiology to achieve active targeted drug delivery systems.

Through precise control of miniaturized robots and ability to control dosage through payload, effective minimally invasive drug administration can be carried out. In order to be used for a drug delivery vehicle, these robots must operate in micro-or nanoscale powered by a wireless source able to navigate in complex biological fluid environment and capable to penetrate soft tissue. Thus, the inspiration to mimic the spiral swimming of the bacteria by directly utilizing the bacterial flagella in the fabrication process described in **chapter 10**.

In any way, the field of tissue engineering has been playing intrinsic and potential roles in replacing or restoring physiological functions or diseased and/or damaged organs. Thus the important role of the scaffold-cells porous architecture that, developed and controlled by a computer-aided design program, becomes functional tissue/organ after implantation. Naturally the selection of polymeric materials, suitable for specific tissue regeneration, is an important challenge in the fabrication of scaffolds. They, in fact, have to be safe, biocompatible, mechanically stable, non immunogenic, and biodegradable ,while their degradation products should remain nontoxic until elimination.

However, successful tissue regeneration is highly dependent on the execution of balance between the physiological and synthetic scaffold properties which have to enhance the cellular attachment, proliferation, and differentiation.

The most important limitations in developing artificial tissues, that mimic the physiological environment, are allowing hierarchical multicellular interactions three-dimensionally under perfusion culture for nutrient supply and waste removal.

By combining biology with bio-MENS technique, such as the microfluidic-based ones, it seems possible to overcome these limitations.

The conclusive chapters are reported on **Part IV** - *Nanotoxicity Studies and Applications in Eco-Biosystems reported* by **chapter 11** - *Environmental Applications of Nanomaterials* and **chapter 12** - *Cytotoxicity of Biosynthesized Nanomaterials and Functionalized Use in Therapy.*

Recent advanced in nanotechnology regarding the environment have been focused on two opposite strands of research: the utilization of nanomaterials in environmental applications and the potential toxic effects of engineered nanoparticles on ecosystems and humans.

The former group pays more attention to the potential of nanotechnology to solve environmental pollution problems by providing innovative tools for monitoring and controlling contaminants in environmental media. Thus the development of nanobiosensors, membranes, adsorbents, and catalysts more sensible for decontamination than the normal environmental remediation technologies which,

due to their enhanced and upgraded properties, show higher sensitivity and specificity.
On the other hand, nanomaterials may be considered as pollutants when released into the environment during the production from manufacturing processes, from products, and from technical compartments. However, the release of nanomaterials from nanomaterial-containing products into the environment, such as atmosphere, soil, and water is considered as a primary release route. Naturally their bioavailability in the environment is of primary importance to evaluate their toxic effects, largely associated with the physicochemical properties of the particles.
Although the progress made in understanding the impact of nanomaterials on the environment, several issues need to be yet solved, such as a more accurate definition of their release, together with the characterization of their behaviour, fate, and bioavailability in the environment. Additionally, while the hybrid nanomaterials composed of nanotubes, nanoparticles, biomolecules and polymers have shown successful results in biomedical applications, their nanosize, increased surface area, solubility, and pH are considered the main causes of their toxicity.
The functionalization of nanomaterials is one of the means to reduce toxicity at the minimal level, enhancing and affecting their cellular activity. Surface nanomaterials' functionalization, in fact, modifying their physicochemical characteristics, enable their self-organization and render them compatible and effective in intra cellular application. Moreover, the consequent increase of solubility and escape from primary immune reactions strengthen the possibility of nanomaterials to be used as carriers of biological and therapeutic molecules.
This book, focused principally on development and application of nanomaterials in the biomedical field, gives an overview on the characterization and preparation of nanoparticles and polymeric matrices assembled and bio-inspired to nature.
Written by an international team of expert in biology and chemistry, it emphasizes the need for a more stringent approach to better integrate chemistry, physics and biology for solving the many aspects of the tissue and organs engineering open until to day.
Offering a good overview on nano-biomaterials as medical devices, drug delivery and tissue engineering systems, the book may be of help not only to specialists on polymer materials, dermatologists, plastic surgeons and clinicians, but also to students in chemistry and medicine and marketing specialists who should like to know more in deep the different interdisciplinary aspects of Nanobiotechnology.

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Trends in Biomaterials

by GP. Kothiyal and A. Srinivasan

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Biomaterials is a multi-disciplinary field involving material and mechanical engineers, biomedical doctors, biologists, chemists, surgeons, regulator affairs and many other scientists interested on life science. This book, organized in 5 chapters, reports the historical perspective, progress and the future trends on synthesis, processing, characterization, properties, and applications of the biomaterials.

Chapter 1 - *Bioactive Glass and Glass-Ceramics Containing Iron Oxide: Preparation and Properties*, reviews the developments in bioactive glasses and glass-ceramics containing iron oxide. In the last 10 years, many interesting studies have been reported in this field regarding the biomedical applications. Glasses with more than 60% silica content, in fact, because of their capacity of accommodating higher magnetic content, have been and would be of great interest for the biomaterial scientists.

The bioactive glass (i.e. bioglass) has been a major advancement in biomaterials because, for example, it may mimic human bone in its interaction with human fluid. Thus, magnetic bioglass and bioglass-ceramics have earned a special place in the area of health care especially in orthopaedic applications, because of their better mechanical strength.

The glassy network structure in fact, can form strong ionic/covalent bonds in three-dimensional space that, depending on its surface area, pore size and surface morphology and naturally of the method of preparation adopted, may play an important role for its specific bioactivity. Thus, the necessity to develop appropriate productive and characterization techniques useful to obtain the right engineered tissue, involving also the use of stem cells, signalling molecules and novel functional biomaterials.

These news are reported and focused on this chapter, exploring the possibilities to make the more smart combinations of biodegradable polymers of interest in biomedicine.

Self- Assembly Approach for Biomaterials Development is the topic of **chapter 2**. Self-assembly is a methodology for making different nanostructured biomaterials to be used for medical applications. By this method it is possible to make structural organizations on scales ranging from molecules to galaxies. It involves non-covalent interaction among amphiphilic molecules to make structure that may be changed by external conditions, such as pH, ionic strength, and solvent. These structures offer an attractive route for encapsulation and controlled delivery of drugs due to their remarkable advantages such as improved drug solubility, enhanced bioavailability, and passive targeting. Unfortunately, most of these self-assembled structures are sensitive to dilution, which limits their usage during practical applications. To limits this problem they may be incorporated in hydrogel matrices having mesh size smaller than the dimensions of the aggregates. Hydrogels are three-

dimensional networks of cross-linked hydrophilic polymer chains prepared by the entanglement of long fibrous polymeric structures in water, made by different methods such as covalent bonds, hydrogen bonding, van der Waals interactions, etc. Thus, different structures such as micellar solutions, micro/nano emulsions, micro capsules, liposomes and many others have been developed for drug delivery applications with the aim to overcome the drawbacks of conventional drug carriers.
By this method, it is possible to minimize drug degradation, prevent harmful side effects, and increase drug bioavailability. For characterizing the self-assembled structures, light scattering techniques are considered the most accessible methods. The role of the natural hierarchical structures and the methods to mimic them for producing tissue of biomedical interest is reported by this chapter also.
Chapter 3 - *New Trends in Bioactive Glasses: The Importance of Mesostructure*, discusses again on the bioglasses as the most promising replacement/regenerative materials for bone reconstruction. This glass material can easily be bond to the bone tissue, stimulating its growth by the action on cell of their dissolution ingredients equally biodegradable. Thus, the chapter reports the last methodologies used to process and control the bioglass hierarchical structures, focusing the debate on the techniques used at this purpose.
Bioglass, in fact, is one if the most attractive synthetic bone replacement materials available being potentially more bioactive than pure calcium-phosphate derived materials. It is typically prepared from high-purity raw materials, and may be produced in a wide range of forms to serve various functions in the body, because of its nanoporose and active structure.
It is to underline that more than 2.2 million bone graft operations are carried out worldwide every year to repair bone-related defects in orthopaedics and dentistry. But it is also to remember that while autografts are the most preferred choice for the treatment of bone defects, their limited supply and donor-site morbidity lead to serious problems. Thus the necessity to find synthetic biomaterials for bone substitutes.
Tissue engineering has emerged as the most common approach for the regeneration and repair of tissues and organs lost or damaged as a result of trauma, injury, disease, and aging.
With the advent of new technologies the science of materials has evolved rapidly using natural biofibers for producing innovative tissues capable to mimic the native extra cellular matrix (ECM). To reach this goal, a properly designed scaffold architecture have been developed by the use of natural polymers such as collagen, silk, cellulose, chitin, starch as well as of synthetic and biodegradable polyesters such as polylactide and polyglicoside biomaterials. At this purpose, these microstructured scaffolds, mimicking the ECM architecture, must have dimensions comparable to the size of cells to facilitate the tissue regeneration. Naturally, the safety aspects of fiber and scaffold related to their biological characteristics such as biodegradability, biocompatibility, and antigenicity are of great importance for their use in biomedical applications.
Thus, the selection of any polymer and fiber should, among many, depend on its degradation time and mechanical properties, providing sufficient mechanical strength without collapsing until the damaged tissue heals completely. Moreover, the micro-nano fibers can also be functionalized by surface and bulk modifications incorporating bioactive molecules, to influence and support the contacting cells.
In conclusion, biomaterials implanted inside the body have to interact well with the native tissue while performing their requisite functions without being physiologically rejected. Thus, while three-dimensional scaffolds support cell-cell and cell-matrix interactions, the diameter of nanofibers pro-

duced by processing techniques such as electrospinning and melt spinning may mimic the ECM fibrils effective for the cell growth. Description of the most used natural and man-made fibers together with the different methodology to produce scaffolds for medical use is the topic focused on **chapter 4** – *Biomaterials based on Natural and Synthetic Polymer Fibers*.

The success of any material intended to be used for *in vivo* medical applications depends on its biocompatibility. The response to any implant debris, in fact, is dominated by local immune activation of macrophages, tuned by the local inflammatory processes connected with biomaterial used, for example, to make the components of an artificial hip prothesis.

Chapter 5 - *Biomaterials in Total Hip Joint Replacements: The Evolution of Basic Concepts, Trends, and Current Limitations-A Review*, reports developments and advances in biomaterials used in total hip replacement (THR). The primary aim of total hip arthroplasty (THA) is to provide a painless, mobile, stable, and durable joint.

Different biomaterials, articulations, fixation principles, and design used for THA are reported and described, highlighting the major contributions given from material researchers and orthopaedic surgeons. Moreover, the influence of material properties on *in vivo* performances and their selection to avoid undesirable effects are discussed, underlining the need for finding ways and means to achieve the best implant stability, necessary for improving its longevity and ameliorating the quality of life of the patients.

This interesting book written by two known scientists, reporting updated and recent research studies on the technological and practical aspects of biomaterials may be of great help for researchers of the chemical and medical communities. Additionally it may be useful for students who wish to understand the connection between the biomaterial production and its clinical use, deepening their engineering perspectives as well as clinical aspects.

P. Morganti Ph.D.
Editor-in-Chief


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Dear Colleagues,

Enriching the knowledge of human and environmental ecosystems will enable scientists, policymakers, businesses, mass media and consumers to better understand the limit and feasibility of industrial sustainability, and respect the criteria of circular and green economy. This is the main objective of the 13th ISCD International Congress. The Grand Opening of the Congress, featuring on the theme of Cosmetic Dermatology for a Green Future: Myth and Reality, is going to be held in Rome University "G. Marconi" – a prestigious Simonetti-Odescalchi Palace - via Vittoria Colonna 11 in 22th of November 2018.

All the topics addressed by well-known and multidisciplinary scientists coming worldwide from Academia and Industry,will be based on the new concept of Precision Medicine.By the use of advanced R&D studies and innovative technologies tailored prevention and treatment strategies will be reported .Thus scientists and attendants will try to understand and map out the interactions between environment and the human unique characteristics, including genome sequence, microbiome composition, health history, life style and diet. For the first time scientists from different fields and experiences from medicine to chemistry will discuss all together the necessity to make totally biodegradable the entire product (content and packaging materials), for maintaining the human wellbeing safeguarding the environment' equilibrium.

In conclusion, all the topics of this congress will report and underline the potential benefits of a green growth based on the production of drugs, food, diet supplements, cosmetics, and textiles made by the use of waste materials with a low consumption of water and energy, and should be useful to create innovative goods and new jobs. So doing it will be possible to preserve the natural raw materials and the planet biodiversity maintaining our well-being for the incoming generations also.

Hong-Duo Chen
President, ISCD

Pierfrancesco Morganti
Secretary General, ISCD

Dear Colleagues,

We are pleased to announce the 2nd INDERCOS-WINTER Congress, held alongside the 13th International ISCD Congress, taking place in Crowne Plaza Roma - St. Peter's, 22 - 24 November 2018 in Rome - ITALY. The main topics of this meeting will be "Cosmetic Dermatology for a Green Future: Mith & Reality". Through plenaries and parallel workshop sessions, we aim to share insights and experiences and discuss how advances in aesthetic dermatology. In order to success this, we have very distinctive international speakers with extensive experience and a range of expertise across aesthetic dermatology. Cosmetic dermatology is growing increasingly common in today's society. From reality television shows to magazine covers and documentaries, the public has never been more aware of the ways that cosmetic dermatology impacts patients from head to toe. Here's the truth behind some common misconceptions about cosmetic dermatology.

The question "Could Dermatology of today be considered to have two distinct camps?" will be debated. The two camps will be considered as follows; on one side, a growing army of cosmetic/surgical dermatologists armed with fillers, Botulinum toxins and lasers with patients happy to pay for these procedures, on the other hand, medical dermatologists which provide traditional therapies to patients. We believe that in the future of Dermatology training a new combination of cosmetic and medical dermatology will form and thanks to this unique training, dermatologists will have the medical knowledge and skill necessary to safely perform many cosmetic procedures. We hope you will be together with us in this fascinating, high quality scientifically educational congress and we look forward to your precious participation and feedback.

Ümit Türsen
Co- President, INDERCOS

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GENERAL INFORMATION

Congress Date and Venue
13th World Congress of the International Society of Cosmetic Dermatology (ISCD) & 2nd INDERCOS Winter Meeting will be held between 22-24 November, 2018 at Crowne Plaza Roma - St. Peter's
Rome - Italy.

Congress Language
The Congress will be held in English.

Congress Badges
Badges must be warn during all sessions, social activities and exhibitions. All participants who have finalized their registration may receive their badges from the registration desk together with all other documents. Those who wishes to register onsite will also receive their badges from the desk.

Accreditation for Continuing Education
The congress will be presented to the approval of Turkish Medical Association Continuing Medical Education Accreditation Board for the "Continuing Medical Education"

Invitation Letter
All registered participants upon request has the right to ask for a letter of invitation. This invitation can only be used to facilitate permission to participate from their institution.

Certificate of Participation
Certificate of participation will be given to all registered participants of the congress. Certificates will be distributed on the last day of the congress.

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REGISTRATİON

CONGRESS REGISTRATION FEES	Until April 7th 2018
Registration Fee	350 Euro

* Registrations made until April 7th,2018 will be considered as early registration
* Cancellations after April 7th,2018 will not be accepted. Name changes are possible.
* All participants and industry representatives must register. Fees include; participation to the scientific program, congress bag and congress abstract book.

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For the registrations to be completed, please transfer the sum of payment to the account details provided below and fax (+90 212 258 60 78) or e-mail (indercoswinter@figur.net) the proof of payment to Figür Kongre ve Organizasyon.

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to be announced...

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Quarterly Review of Cosmetic Dermatology

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Contents

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Correction of defects in the flaws of Bone Tissue Facial, through the use of Tricalcium Phosphate or Calcium Hydroxyapatite with Plasma Gel. A Clinical Assessment Study

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Received: *October, 2017*

Key words: *Allografts; Antiaging agents; Chronoaging; Zygomatic; Malar and maxillary bone reconstruction; Autologous growth factors; Bone substitutes; Ceramics; Demineralized bone matrix (dbm); Hydroxyapatite; β tricalcium phosphate (β-tcp);*

Summary

The aging process as it is observed by all patients begins after the first third of life and involves all the tissues, even for the depletion of adult stem cells responsible for the reintegration of lost tissues. The epidermis and dermis tend to thin, fatty tissue loses consistency, and bone tissue changes. The impoverishment of the tissue layer leads to typical wrinkles and folds that can be observed on the face, typically as early as 40 years. The purpose of this study was to clinically evaluate the volume increase near zygomatic and maxillary bone by using tricalcium phosphate powder or calcium hydroxyapatite combined with a gel derived from autologous plasma in order to contribute to the attenuation of the anesthetics of bone hypovolumetry.

The hypothesis of scientific work was that by depositing exclusively injecting a plasma compound with calcium hydroxyapatite or tricalcium phosphate at the zygomatic, malar and maxillary levels, taking care to scratch the periosteum with the tip of the needle, bone to supplement what deposited through the injection, it could have a longer duration and three-dimensional effect of aesthetic correction together with an integration with the bone itself, unlike what happens with absorbable fillers now in use.

All volunteer patients have been studied after obtaining regular informed consent. Patients were photographed at 0, immediately after treatment and after four months demonstrating the perfect integration of injected material in the absence of pathological inflammatory responses.
This study, launched in November 2011, showed that tricalcium phosphate and calcium hydroxyapatite combined with an autologous gel derived from platelets is perfectly tolerated as biomaterial in the volumetric integration of bone tissue.

Riassunto

Il processo di invecchiamento cosi come viene percepito dai pazienti inizia dopo il primo terzo della vita e coinvolge tutti i tessuti, anche per il depauperarsi delle cellule staminali adulte deputate al reintegro dei tessuti persi. L'epidermide ed il derma si assottigliano, il tessuto adiposo perde di consistenza ed i tessuti ossei si modificano. L'impoverimento dello strato dei tessuti porta alle tipiche rughe e pieghe osservabili sul viso, in genere già verso i 40 anni. Lo scopo di questo studio è stato quello di valutare clinicamente l'aumento volumetrico in prossimità dell'osso zigomatico e malare effettuato mediante l'ausilio della polvere di fosfato tricalcico o di idrossiapatite di calcio uniti ad un gel derivato dal plasma autologo al fine di contribuire all'attenuazione dell'inestetismo delle ipovolumetrie ossee.
L'ipotesi di lavoro scientifico era quella che depositando con metodo esclusivamente iniettivo un composto di plasma unito ad idrossiapatite di calcio o fosfato tricalcico a livello zigomatico, malare e mascellare, avendo cura di scalfire il periostio con la punta dell'ago e permettendo all'osso di integrare quanto depositato attraverso l'iniezione, si potesse avere una durata maggiore nel tempo e tridimensionalmente della correzione estetica unitamente ad una integrazione con l'osso stesso, diversamente da quanto accade con i filler riassorbibili oggi in uso.
Le pazienti sono state fotografate a tempo 0, immediatamente dopo il trattamento e dopo quattro mesi dimostrando la perfetta integrazione del materiale iniettato in assenza di risposte infiammatorie patologiche.
Lo studio ha permesso di dimostrare la perfetta integrazione delle ceramiche con i tessuti

INTRODUCTION

The face aging that is observed after the first third of life is characterized by a progressive intensification of folds and relaxations caused by the loss of tissue (1), especially at the upper corners of a hypothetical triangle, called beauty, which have the apices the zigomatic area.
This process also includes the remodeling of zygomatic and maxillary bone that contribute to changes in the skull structure. In fact, age depletes not only the dermis and the subcutaneous tissue but also the bone (2).
Bone resorption significantly increases the typical appearance of time on the face, especially at zygomatic, maxillar and mandibular levels, affecting negatively the hypothetical but characteristic geometric formation (3). Patients in whom these signs of time may be evident may be aesthetic benefit from treatment with tricalcium phosphate or calcium hydroxyapatite injected directly in contact with this bone formulation as a starting point for subsequent epidermal, dermal and hypodermic integration.

MATERIALS AND METHODS

This study started on November 7, 2011 with the first session and I used a microporous hydroxyapatite with granules of between 70/ 100 microns or a microporous tricalcium phosphate with 30/40 micron granulation.
The choice of the implant to be used on patients was quite random. It pushed me to use these materials on the skull bone structures, to certify that the inorganic component of all mineralized tissue tissues, such as bone, is predominantly composed of tricalcium phosphate salts or hydroxyapatite, together with other inorganics such as carbonates calcium and sulphates present in inferior amounts and perfectly biocompatible (5) (12) and it is known that bone weight is represented by 60-70% of calcium hydroxyapatite.
Differences between the two materials can be identified in their chemical composition: tricalcium phosphate has a calcium/phosphate ratio of 1.5 and has a crystalline structure while hydroxyapatite corresponds to tribasic calcium phosphate with a calcium/phosphate ratio of 1.67 equal to bone mineral. In maxillofacial surgery, hydroxyapatite has been and is used in the form of shaped blocks in bone defects, in the post traumatic reconstruction of the face, especially of the orbital, zygomatic and maxillary bone portions. It is also used as a block or as a powder.
The ability of hydroxyapatite to bind to bone has been demonstrated in several studies, as well as its biocompatibility and peculiar properties to promote cell differentiation to form new bone (3,5,6,7). No toxicity or flogistic phenomena have been detected at clinically relevant positioning or remotely, and perfect biocompatibility has also been demonstrated for tricalcium phosphate in soft tissues without epidermal or dermal inflammatory inflammation (18), exactly as reported for calcium hydroxyapatite (9,10,11).
Scientific opinions on calcium hydroxyapatite resorption are contrasting, while some authors in their studies have confirmed that, unlike tricalcium phosphate, it does not respond to absorption (11,12,13), other authors have argued that it also goes meeting the same resorption typical of tricalcium phosphate (5,14,15,16,17). But anyway, we can certainly assert that both materials are absorbable.
In this study, I could personally observe a slower clinical development of hydroxyapatite reabsorption than tricalcium phosphate, but better solubilization of the latter in the plasma, though both can be perfectly utilized by the procedure described.

Procedure

For the solubilization of tricalcium phosphate powders and calcium hydroxyapatite contained in ampoules and sterile, 2 cc of plasma derived from 10 cc of blood from a vein extraction supplemented with 0.5 cc of sodium citrate was used to prevent clotting and centrifuged.
Once red blood cell separation is obtained, the plasma in the amount of 2 cc was mixed with the materials studied directly in ampoules containing 500 mg of product, strictly observing sterility and aseptic charges. After solubilization inside the ampoules, the materials were taken with a syringe and placed in a dry sterilizer for the time and temperature required for coagulation. Subsequently, the compound thus obtained was passed several times within a two-way tap to achieve its homogenization and distribution as much as possible.
The plasma gel coupled with the ceramics thus obtained was injected to patients via a 21G or 22G needle on the malarial, zygomatic and maxillary anatomic areas very slowly and taking care to scratch the periosteum during injection. Particular attention was paid to the execution, to the infrared, zygomatic and facial holes, and to the mental hole, whose shuttering with the material of the study would have had repercussions at the level of nervous, sensory and motor endings on the facial musculature (Fig. 7). Detailed informed consent was obtained and patients photographed before and after treatment. The average age was 51 years.
Checkups and evaluations were carried out after 15, 30, 60, 90 and with a 120 day follow-up visit. The degree of correction obtained for the treatment was objectively evaluated using an analog scale 0-10 (0= no correction; 5= satisfactory correction; 10= total correction). The degree of satisfaction and effectiveness was obtained subjectively, asking patients if there was an itching, pain, or burning sensation during and after the procedure.
All patients reported a total correction of 15, 30 and 45 days and a very good 120-day correction. The best parameters were observed in patients treated with calcium hydroxyapatite.
All patients stated only a burning sensation during the injection of a few seconds. No one has stated a foreign body sensation or pruritus reported clinical details worthy of being interpreted as referring to the procedure under study (Fig. 1-2-3-4-5-6)

RESULT

All patients were satisfied with the treatment received. Specifically, the three-dimensional increase in volume and soft tissue support has been highlighted. I can say that induction treatment of neocollagenogenesis for the formation of a fibrous capsule in injection sites with this method has yielded clinically relevant results between the very good and the excellent, as the photos show (Fig. 1-2-3-4-5-6).
Through the traumatic scratching of the periosteum by means of the needle tip, it is possible to integrate the ceramics contained in the gel and the bone tissue; gel obtained through plasma thermal coagulation combined with hydroxyapatite or calcium phosphate. Soft tissues in contact with tricalcium phosphate supplemented a prolonged stimulatory effect on fibroblasts with induction of a fibrosis so as to activate a three-dimensional correction of deep wrinkles or slides (17) close to the zygomatic-malarial or maxillary bone.

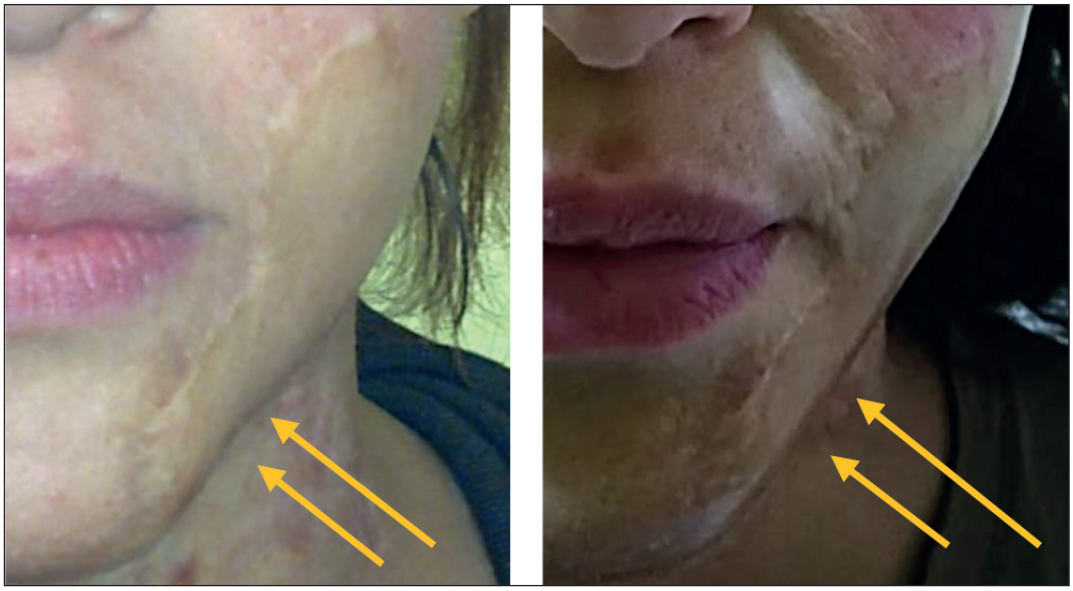

Fig. 1 *Correction with deposition of tricalcium phosphate on the lower edge of the outer face of the mandible (mandibular angle).*

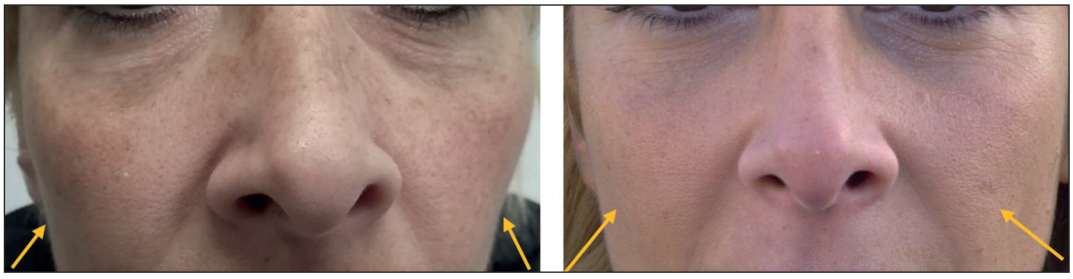

Fig. 2 *Correction of the zygomatic arch, the lateral face zygomatic bone and the zygomatic bone process maxillary level with deposition of calcium hydroxyapatite, correction to 60 days.*

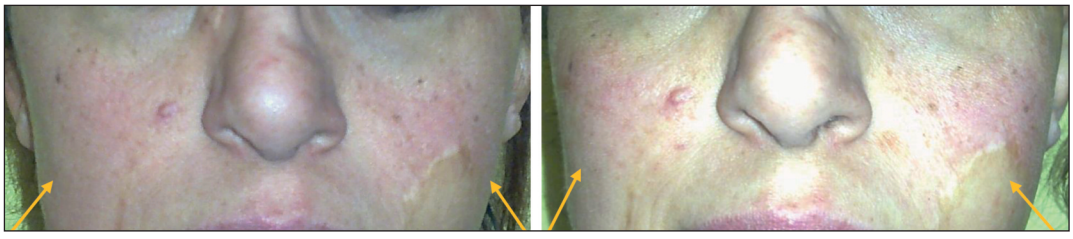

Fig. 3 *Zigomatic area correction with deposition on the side of the zygomatic bone and at the level of the zygomatic process of the maxillary bone with calcium phosphate, 60-day correction.*

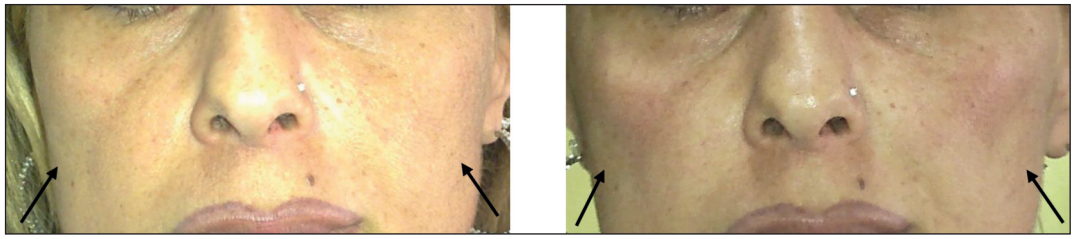

Fig. 4 *Zigomatic area correction with deposition on the side of the zygomatic bone and zygomatic process of the maxillary bone with calcium phosphate, 45-day correction.*

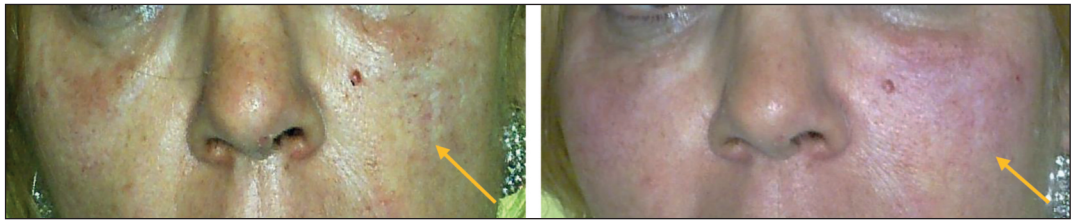

Fig. 5 *Correction of zygomatic area with deposition of tricalcium phosphate on the lateral side of the zygomatic bone and at the level of the zygomatic process of the maxillary bone. Time 0.*

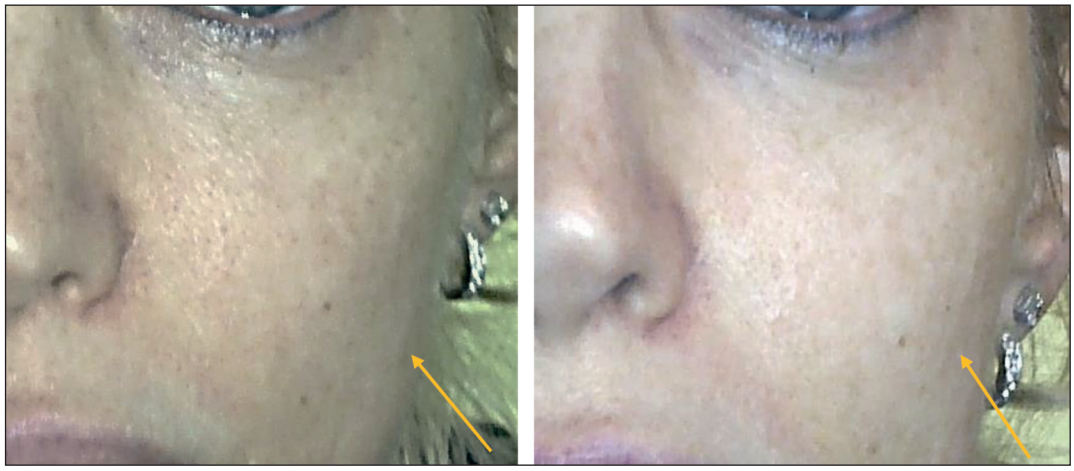

Fig. 6 *Correction of the zygomatic arch, the lateral face zygomatic bone and the zygomatic bone process maxillary level with deposition of calcium hydroxyapatite, correction to 120 days.*

DISCUSSION

Since the late 90's, dermo-epidermal and adipose treatment has also been treated with growth factors derived from plasma derived platelets for centrifugation of venous removal. For these indications there are many extraction protocols that involve different procedures such as that related to the radius of the centrifuge, the tube used or the possessed medical devices, but the general

principle is to separate the red part from the plasma and obtain a centrifuge containing three sections: PRP, Plasma Platelet and Poor Plasma Platelet.

At the base of PRP we find the buffy coat consisting of a more nebulous honey, the richest of platelets and leukocytes. Low density lipoproteins (LDLs) that interact with fibroblasts receptors and activate other stimulatory pathways float at the top of PPP, and should also be used in regenerative treatments.

Growth factors for pathology have been used for years to promote and accelerate the healing of both acute open wounds and chronic lesions, such as in the case of the decubitus, and to accelerate functional recovery related to locomotor functions such as tendinopathies and injuries of professional athletes. They are located within the alpha granules contained in the platelets. They include, IGF-1, PDGF, bFGF, TGFbeta 1, TGF alpha, VEGF.

The cell target of these GFs in aesthetic and regenerative medicine can be recapitulated as follows: VEGF directed towards dermal papilla, EGF towards outer follicular sheath, FGF towards fibroblasts and keratinocytes, TGF beta1 and TGF alpha towards dermal papilla, fibroblasts and keratinocytes, PDGF towards fibroblasts. Not only, in dense granules of platelet there are serotonin, histamine, ADP, and ATP, which of particular importance in regenerative medicine .

The use of PRP dentistry was proposed by Dr. Anitua while as a biostimulant for dermis and epidermis by Dr. Victor Garcia. Dr. Scarano has proposed a mix with tricalcium phosphate for soft tissue treatment.

Here the plasma gel was considered as a vehicle for positioning both calcium hydroxyapatite and tricalcium phosphate powder on the bone tissue by means of a deep injection, as a three-dimensional support without any conditioning on the tissues of GFs (20).

In fact, it must be emphasized that both the time it takes to prepare the plasma with tricalcium phosphate powder or hydroxyapatite powder up to its injection, and to heat it up within the sterilizer for the time required to coagulate the plasma to have the gel produces a total ineffectiveness of the GFs on the tissues surrounding the injection site, as the protein denaturation that already occurs at 41 degrees is abundantly exceeded by the 80 degrees required to form the gel, making them no longer active on the tissues. It is therefore presumed that no effect can be induced by the growth factors present in platelets on the surrounding tissues, since they have been rendered ineffective during plasma gelling.

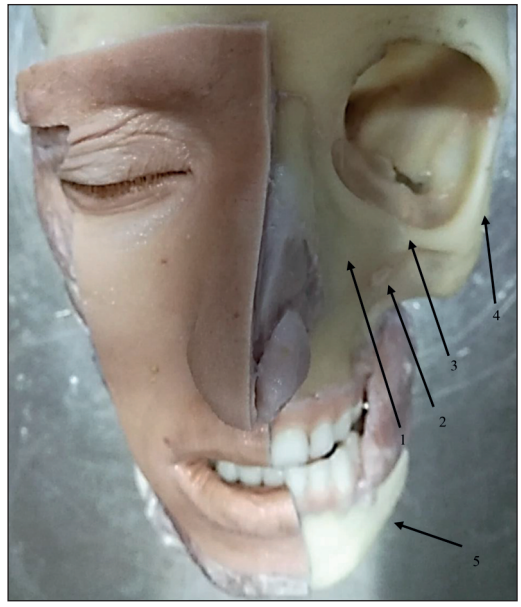

Fig. 7 *Ceramic Positioning Areas: 1-3-4-5. Forum Infraorbital: 2.*

Declaration

For this publication I declare that there is no conflict of interests.

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Biodegradable polylactide/chitin composite fibers: processing, structure and mechanical properties

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Summary

Monofilament fibers based on Polylactide (PLA) and PLA filled with various chitin nanofibrils (CN) were processed by using a twin-screw extruder. Orientation of composite fibers was carried out.
The phase morphology of PLA-chitin composites was observed by scanning electronic microscopy. Rheological and thermal properties of composites were studied.
The influence of the CN types on the mechanical properties of oriented and non-oriented fibers was studied. The type of CN, which are most promising for obtaining a highly oriented fiber, was installed.

Riassunto

Sono state lavorate ed estruse fibre naturali formate da acido polilattico (PLA) e nanofibrille di chitina (CN) verificandone successivamente l'orientamento nel composito ottenuto. La relativa morfologia è stata verificata con la microscopia elettrica a scansione (SEM) studiando anche le proprietà reologiche e termiche dei compositi.
E' stata così controllata l'influenza esercitata da CN sulle proprietà e l'orientamento delle fibre.
In conclusione si è visto che CN è molto utile per ottenere un elevato orientamento delle fibre.

Abbreviations: PLA – polylactide; CN – chitin nanofibrils; LG – lignin; PEG – polyethylene glycol.

INTRODUCTION

Biodegradable polymers have increasing interest over the past two decades in the fundamental research as well as in the chemical industry. The practical value of biopolymers is determined first of all by their ability to biodegradability and biocompatibility. However, only a few types of polymers have been developed as candidates for medical applications, primarily because of their biocompatibility requirements. These polymers include, particularly, polylactide (PLA), polyglycolide (PGA) and their copolymers, poly(e-caprolactone) or some natural polymers such as chitin, chitosan and collagen. Among these polymers PLA type polymers have been applied widely for medical application because of their good biocompatibility, biodegradability, high mechanical strength, and excellent processability by using melt technology. Within the state of current PLA research, many studies have been performed to synthesize PLA with controlled structure and properties. PLA can be easily processed by conventional processing techniques used for thermoplastics like injection moulding, blow moulding, thermoforming and extrusion (1).

A large number of investigations have been carried out on PLA and its copolymers in biomedical applications for resorbable medical implants in the shape of rod, plate, screw, fiber, sheet, rod, sponge, beads for bone and tissue engineering, microsphere for drug delivery system (2, 3). Optimal physico-mechanical properties and shorter degradation time of PLA and its copolymers are generally preferred in such pharmaceuticals applications.

One of the ways to improve mechanical and other physical characteristics is to incorporate some stiff filler phase into the polymer matrix. Often, it was pointed out that the reinforcing phases of the biopolymer is not biodegradable and biocompatible (4), thereby making such composite material medically unsuitable. There exist several biopolymers from which biocompatible nanofillers can be created.

Chitin and chitosan offer a unique set of characteristics: biocompatibility, biodegradability to harmless products, nontoxicity, physiological inertness, antibacterial properties, heavy metal ions chelation, gel forming properties and hydrophilicity, and remarkable affinity to proteins (5). Owing to these characteristics, chitin- and chitosan-based materials, as yet underutilized, are predicted to be widely exploited in the near future especially in environmentally benign applications in systems applied in biological environments.

PLA composites and nanocomposites have been well studied in literature (6, 7), however little record exists for PLA-chitin nanocomposites (8). The existing literatures are largely only relevant to biomedical application dealing with solution blended chitosan-PLA composites (9-11). Thus the application of chitin nanofibrils (CN) for the purposes of mechanical and physical improvements utilizing scalable manufacturing processes is a very important area to explore (3,12,13). Composite materials based on PLA/CN can be of great interest as surgical suture materials, but research work in this area is relatively little.

The aim of this work was to create fibers based on PLA and different types of CN by melt technology. To study the influence of types of CN on the physical and mechanical properties of composite fibers. To process highly oriented fibers based on PLA and CN could be promising for future use as surgical suture materials.

MATERIALS AND METHODS

Materials

Matrix: Semi-crystalline Poly Lactic Acid (PLA), Sinobiom™ PL50, was supplied by Sinobiom, China. The density is 1.25 g/cm^3.

Nanofillers: Chitin nanofibrils of three types (Mavi Sud s.r.l, Italy) were used.

1) Chitin nanofibrils (CN) was obtained by lyophilization of the initial chitin suspension. As a result, the powder consisting of the microparticles was obtained. Figure 1 represents the SEM micrographs of the material taken at different magnifications. It is evident that the microparticles have a strip morphology with the characteristic width of about 30μm and the thickness of 0.1μm (Fig.1a); they consist of nanofibrils with the width of 20 nm and the length in the range of 600–800 nm (Fig.1b).
2) Chitin nanoparticles-biolignin (CN-LG) was done adding slowly a 2% water solution of purified biolignin at pH 12(w/w) into a 2% water suspension of chitin nanofibrils(w/w) at pH 1.9.
   After a mixing of 1 hour at a temperature of 50-60 ºC, PEG is added slowly, mixing for a ~1 h. When at room temperature, the suspension was spray dried to obtained a dark brown powder consisting of the particles with a shape close to spherical. Modified nanoparticles have size in the range from 10 μm to 60 nm (Fig.2a).
3) Chitin nanoparticles modified with PEG (CN-PEG). was done at a 2% water suspension of CN (pH 1.9) neutralized at pH 7 by a 10% water solution of NaOH, is slowly added a 2% water solution of PEG at room temperature by a slowly mixing. Soon after the suspension is spray dried to obtain a light brown powder consisting of the spherical particles with diameter in the range from 10 μm to 67 nm (Fig.2b).

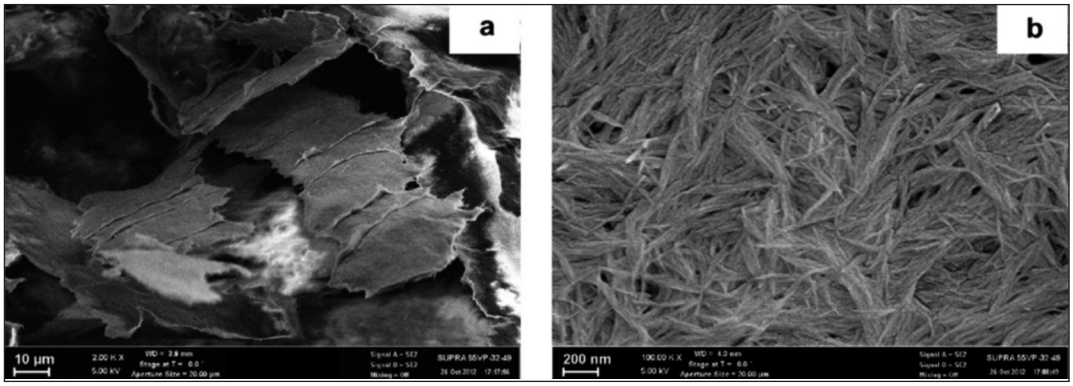

Fig. 1 *Scanning electron micrographs of the film obtained from chitin nanofibril water dispersion by freeze drying (a and b).*

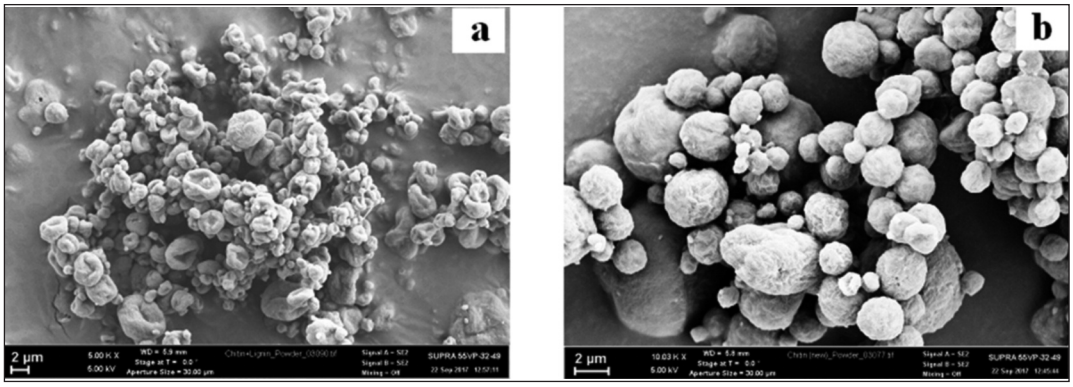

Fig. 2 *Scanning electron micrographs of the powder of chitin nanoparticles modified with biolignin (a) and PEG (b).*

Methods

Processing of nanocomposites

CN and oven-dried PLA were mixed on a 5 mL double-screw microextruder (DSM Xplore, Netherlands) at the screw rate of 50 min^{-1} and temperature 220°C.

In order to minimize thermal degradation of both components, the mixing time was kept low as 5 min. At the exit of a Spinneret with a diameter of 1 mm after mixing, the melt was cooled with a jet of compressed air was supplied to the coil receiver placed at a distance of 260 mm from the extruder and rotating at a speed of 20 min^{-1}. As received monofilament was subjected to orientation drawing by four times ($\lambda = 4$) at the temperature 65±5°C with the use of special equipment. The resulting PLA-based composite in the form of non-oriented and oriented fibers were obtained.

Chromatography

The molecular weight (MM) characteristics of the polymers were determined by the method of liquid-liquid chromatography using a liquid chromatograph Prominence (Shimadzu) equipped with a refractometric (RID-10A, Shimadzu) detector. The HR 4E Styragele column, (Waters, 5 μm, 7.8x300mm) was thermostated at 40 °C. THF was used as a mobile phase, the flow rate was 0.5 mL / min. Polystyrene standards (Agilent Technologies) were used to calibrate the column. THF was distilled over KOH and stabilized by adding 0.02% BHT.

Thermal methods

The thermal analysis of polymer was performed by the TGA and DSC methods. The TGA measurements were carried out on a Netzsch TG 209 F1 Iris analyzer (Germany) in an inert medium in the temperature range of 30–600 ºC at the heating rate of 10 K/min. The DSC analysis of copolymer was also performed in an inert medium on a Netzsch DSC 204 F1Phoenix analyzer (temperature range of –80 to +280 °C, heating rate of 10 K/min). The DSC method was used to determine the glass transition, crystallization and melting temperatures.

Rheological studies

Rheological behaviors of pure polymer and PLA-based composite were investigated by using a Physica MCR-301 rheometer (Anton Paar, Austria), with a cone–plane metering unit (25 mm in diameter, angle of 2º) All measurements were performed in shear mode at 220°C and a frequency of 0,1 s^{-1}. The temperature range of tests was chosen on the basis of the data of TGA and DSC.

Mechanical properties

The mechanical properties of PLA monofilaments prepared by melt spinning were studied on an Instron 5943 tensile test machine. The sample base length was 100 mm and the extension rate was 50 mm/min. From stress-strain diagrams of the samples, their strength σ_b, Young modulus E_0 and elongation at break ε_b, strength in a simple knot σ_{knot} were determined.

Microscopy

SEM images of the cryocleavage surface of PLA/CN monofilaments were recorded on a Carl Zeiss Supra-55 scanning electron microscope (Germany). All specimens were gold coated prior to observation.

RESULTS AND DISCUSSION

Molecular mass of PLA was estimated by using

chromatography method. Number average mass (M_n) of PL50 is 80 kDa and weight average mass (M_w) is 42 kDa, polydispersion PD=1,76.

The technological parameters of PLA processing were selected by using TGA, DSC and rheological analysis. Thermogravimetric and DSC curves is showed on figure 3. The temperature of beginning of weight loss of PL50 is 290°C (Fig. 3, curve I, point 1).

DSC curve (Fig.3, curve II) show glass transition temperature Tg=70°C (fig.1, point 2) and a distinct endothermic peak at 180°C (Fig.3, point 3) of PL50 can be attributed to the melting point (T_m) of the PLA crystallites (14, 15).

Chromatography and thermogravimetric analysis showed that PL50 is pure materials without impurities and suitable for processing by melt technology. Processing temperature of the polymer should be around 220 °C.

The stability of the rheological characteristics of the PLA melt in time in the atmospheric medium at this temperature were investigated (Fig.4, line 1). It is shown that viscosity decreases from 6500 to 1800 Pa•s during 30 minutes.

This imposes a limitation on the processing time of the PLA at least 10 minutes.

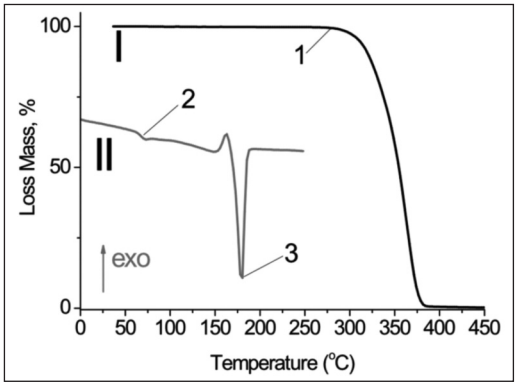

Fig. 3 *Thermogravimetric (I) and DSC (II) curves of PL50: 1-temperature of beginning of weight loss; 2-the glass transition temperature; 3-melting temperature.*

Further melt spinning of PLA composite monofilaments was processed on a twin-screw microextruder with regard to the set technology parameters of material processing.

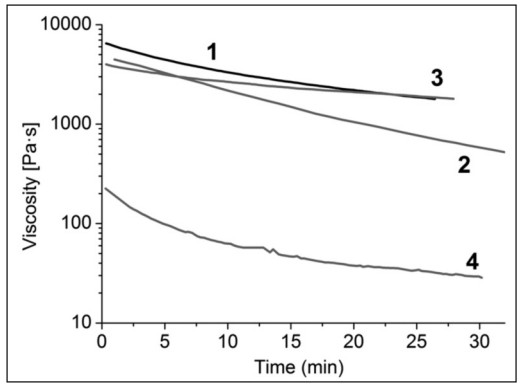

Fig. 4 *Dependence of viscosity of pure polymer and PLA-based composite on time at the 220 °C: 1 - pure PLA; 2 – PLA+1%CN-PEG; 3 – PLA+1%CN; 4 – PLA+1%CN-LG.*

Structures of PLA–chitin composites

To investigate the phase morphology of PLA/CN blends, fractured surfaces at room temperature of the blends were observed by SEM after being gold coated. As shown in figure 5, chitin particles of all three types are evenly distributed in the PLA matrix and retain their original dimensions and shape. But in the case of CN, some particles are combined into conglomerates with size up to 20 μm. As seen, it was not possible to achieve dispersion to the nanoscale level of the part of CN.

Thermal properties of PLA–chitin composites

TGA curves for different types of chitin were carried out to evaluate their degradation profiles and thermal stability (Fig. 6.). In the thermogram of CN-PEG, there was one degradation step, which starts at τ_5=335 °C, determined by the derivative TGA curves. TGA curve for CN sho-

wed the start thermal degradation nanofiller at τ_5=250 °C. CN-LG showed a combination of thermal degradation of lignin and chitin at the temperature of 210 and 340 °C respectively [16, 17]. As mentioned above, the processing temperature of the composites is 220 degrees, which can lead to the destruction of the filler, as well as to the destruction of the polymer matrix. Also it is seen (Fig. 6) the mass losses for CN-LG and CN at the temperature of τ_1=100 °C. Thus, these fillers must be dried before mixing with PLA with used the extruder for removing of water.

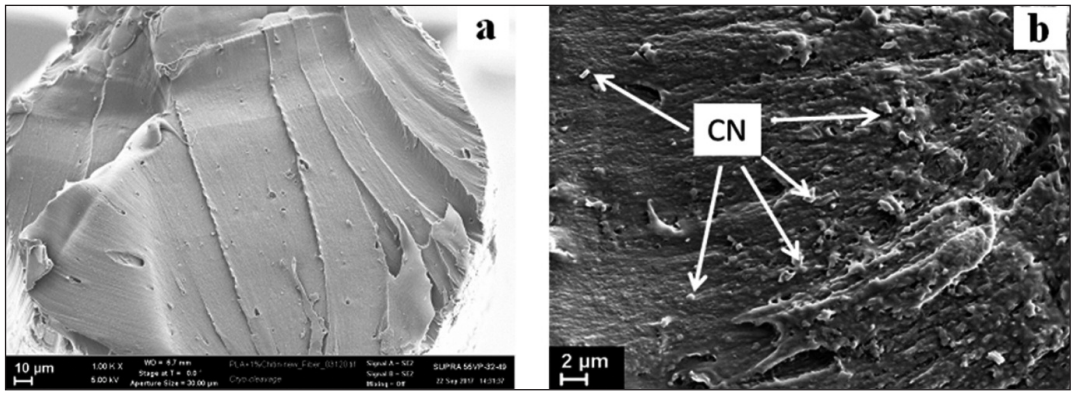

Fig. 5 *Micrograph of fractured surface of PLA+1%CN.*

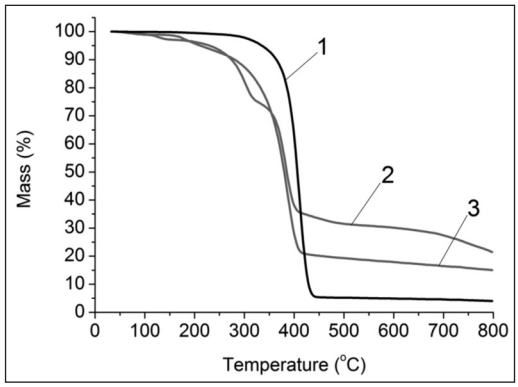

Fig. 6 *Thermogravimetric curves of different types of chitin: 1 – CN-PEG; 2 – CN-LG; 3- CN.*

DSC scans were performed for three PLA/CN composites are presented in figures 7 and 8.

The results (Fig.7) indicated that CN and CN-PEG does not significantly influence on the glass transition and melting behavior of PLA, which were at 62°C and 180°C. But PLA/CN-LG blend has double melting peaks at temperature 160 and 168 °C. Fig. 8 illustrates that chitin in any form except for chitin, modified lignin, can serve as a crystal nucleating interface, increasing the crystallinity from 8,6 J/g for pure PLA to the 11,8 and 37,9 J/g for PLA filled with CN-PEG and CN, respectively (18). In addition, only the CN content has influence on the crystallization temperature which is slightly increased up to 105°C, while for the pure PLA it is 96°C (Fig. 8, line 1).

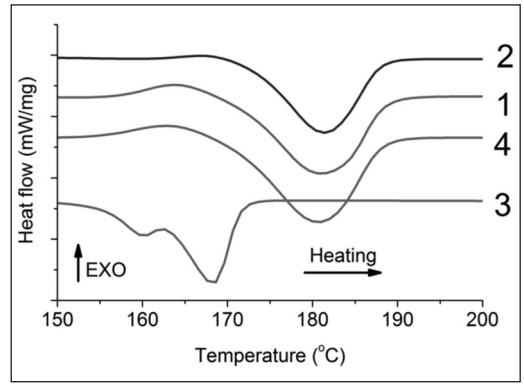

Fig. 7 *DSC curves of PLA/Chit composites (2 scan): 1 – pure PLA; 2 – PLA+1%CN; 3 - PLA+1%CN-LG; 4 - PLA+1%CN-PEG.*

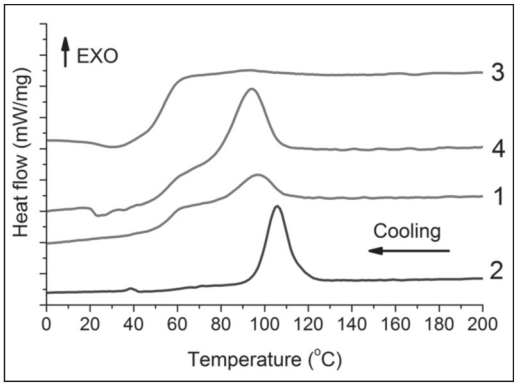

Fig. 8 *DSC curves of PLA/Chit composites (cooling): 1 – pure PLA; 2 – PLA+1%CN; 3 - PLA+1%CN-LG; 4 - PLA+1% CN-PEG.*

Rheological behaviors of PLA–chitin composites

According to the results of rheological analysis (Fig. 4) it can be noted that a melt of PLA with CN-LG has the lowest viscosity of about 200 Pa·s compared to other composites. It decreases in the course of the experiment due, probably, to destruction of the CN-LG particles during its dispersion in the polymer melt at temperature of 220°C (see TGA curves of chitin fillers (Fig.6). This destruction of CN-LG can decrease molecular weight of PLA in turn.

The incorporation of CN and CN-PEG into PLA do not increase the melt viscosity of the composites and their viscosities are about 4000 Pa·s. It should be noted that the viscosity of PLA filled with CN-PEG is reduced over time, and filled with CN is almost stable. Viscosity of PLA filled with CN-PEG decreases up to 400 Pa·s during 30 min of test, while viscosity of PLA-CN decreases only up to two times (η=1800 Pa·s).

It is known that if the dispersion of the filler to the nano-sized particles is the sharp increase of melt viscosity at low shear rates, the reason for this is the formation of a structure (percolation) in the grid of nanoparticles in the volume of the polymer matrix. In this case, the chitin fibrils likely failed to destroy up to nanoscale level. Further work will be carried out to improve the technology of dispersion chitin nanoparticles in the melt PLA.

Tensile properties of PLA–chitin composites

The results of mechanical tests of the non-oriented PLA/CN composites are presented in Table I. As can be noted from Table I particles CN in the quantity of 1% do not influence practically on the mechanical properties of the composite fibers and all mechanical parameters remain at the level of pure PLA taking into account the error: tensile strength ~ 50 MPa, Young modulus ~ 2.6 GPa, and elongation at the break remains at the level of 100%.

TABLE I

Mechanical properties of non-oriented PLA–chitin nanofibrils composites

Parameter	PLA	PLA+1% CN	PLA+1%CN- LG	PLA+1%CN- PEG
Tensile Strength, MPa	55±10	45±8	65±17	36±1
Young modulus, GPa	2,6±0,1	2,6±0,2	4,0±0,2	3,0±0,1
Elongation at the break, %	100±50	80±60	3,1±0,8	1,6±0,2

Fibers with the addition of CN-LG have a strength slightly higher than CN (σ_b=65 MPa), but they are brittle with deformation up to a break of 3%. Young modulus of this composites is higher than pure PLA (E_0=4 GPa). At the same time monofilaments with CN-PEG has also brittle deformation up to a break of 1%. The strength of these monofilaments is lower than that of the initial 36 MPa.

The elastic modulus is slightly higher than pure PLA (E_0=3 GPa). Thus, the introduction of CN-LG and CN-PEG leads to the embrittlement of PLA fibers. Only composites with CN are suitable for further high-temperature orientational draw by 4 times (λ=4).

Mechanical properties of oriented fibers based on pure PLA and PLA+1%CN are shown on Figure 9 and in Table 2.

Figure 6 shows representative stress–strain curves for oriented PLA and PLA–CN fibers containing 1% Chit. It can be noted (table 2) that the addition of 1% CN nanoparticles reduces the strength of composites fibers compared with pure PLA. So the strength of PLA+1%CN is 246 MPa, while pure PLA have σ_b = 274 MPa. It can be seen that at 1% CN, The Young modulus of the composites and elongation at the break slightly decrease (table II).

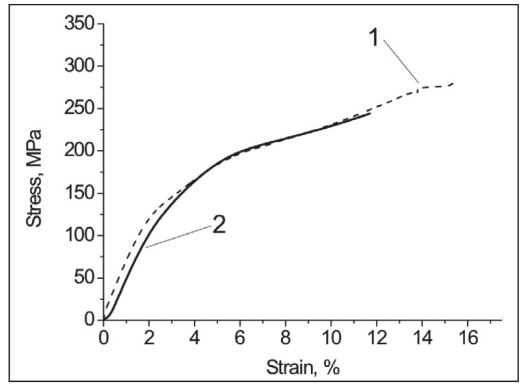

Fig. 9 *Stress–strain curves of oriented fibers based on PLA (1) and PLA+1%CN (2).*

The data values of the filler concentration is likely to impede the realization of the orientation process in the polymer matrix and reduce the likelihood of the formation of structures responsible for the mechanical properties of the material, which leads to a drop in the strength of composite fibers.

Composite fibers based on polylactide with chitin can be used as suture surgical materials. Strength in the knot is one of the indicators of quality material of suture yarns.

TABLE II

Mechanical properties of oriented fibers based on pure PLA and PLA+1%CN.

Parameter	**PLA**	**PLA+1% CN**
Tensile Strength, MPa	276±20	246±4
Young modulus, GPa	6,2±0,3	5,7±0,1
Elongation at the break, %	16±4	12±1
Strength in a simple knot, MPa	168±40	179±26

Comparison of the strength in the knot of oriented fibers based on pure PLA and filled with 1% CN is presented in table 2 also. It is seen, the strength of the fibers in the knot decreases in the comparison with the tensile strength by 40 and 30%, respectively, for pure PLA and PLA/CN.
The introduction of chitin fibrils does not lead to a decrease in the strength in the knot, which remains at the level of a pure PLA. The spread of values is within the measurement error. Such a result is extremely important in the development of surgical composite materials (fibers, yarns), the level of mechanical properties of which is primarily estimated by the strength of the knot.

CONCLUSION

As a result of the work, composite materials were obtained in the form of fibers based on PLA and various chitin particles (CN, CN-PEG and CN-LG) in the concentration 1wt%.
The phase morphology of PLA–chitin composites was observed by scanning electronic microscopy (SEM). Nanoparticles are uniformly distributed in the PLA matrix, but some particles united in conglomerates the size of 20 μm. It should be noted that the viscosity of PLA filled with CN is almost stable at the time compared with pure PLA and other composites.
The addition of chitin particles modified by lignin leads to the degradation of the PLA during processing by melt technology. Addition of CN and CN-PEG particles to a polymer induces heterogeneous crystal nucleation resulting in increased crystallinity.
The influence of the type of chitin particles on the mechanical properties of oriented and non-oriented fibers were studied. It is shown that introduction of 1% CN-LG and CN-PEG make the composites fibers brittle, while mechanical characteristics of non-oriented fibers PLA filled with chitin remain at the level of pure PLA.
Promising composite fiber PLA+CN are suitable to orientational draw. Such high oriented fibers may be promising for the development of surgical suture materials with regulated physical and mechanical properties.

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Scenarios in the Cosmetics Industry

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Cosmetic Industry Evolution

In 2016 the Italian cosmetic industry once again confirms the inelastic and anti-cyclical nature of the Italian cosmetics sector in the context of the country's manufacturing industry. Indeed, in what has been another tough year for consumer goods, the cosmetics industry turnover, the value of production in other words, has seen an increase of 5.3% in comparison to the previous year, for a value exceeding 10,502 million euro. This is despite persisting sluggish trends in the domestic market.

The cosmetics industry therefore demonstrates greater robustness compared to other sectors within the Made in Italy system, including in financial terms, as confirmed by the new project for the analysis of the sector's financial statements: the financial and operational values in particular have been highlighted, and in general terms, these enable the sector to face a political and economic scenario which remains very uncertain.

Sales channels are undergoing a slow yet inexorable transformation, already experienced abroad but which is now being felt in Italy too. For this reason, domestic demand has had a mildly positive impact on production volumes, with growth of 0.7%, equivalent to 6,209 million euro. The domestic market no longer appears to be conditioned by consumer purchasing behaviours.

Despite paying closer attention to the purchases they make than they once did, consumers nonetheless refuse to make sacrifices with regard to cosmetics and personal care, instead graduating towards new distribution methods and more advanced product types, whilst keeping a closer eye on prices.

A breakdown of sales on the domestic market shows positive trends in direct sales, above all thanks to e-commerce - indeed, this sector has grown by 7.8%, while others channels struggle to recover: sell-ins have witnessed a slow increase, equivalent to +0.9% in perfume shops, while pharmacies (-0.1%) and organised mass market retailers (-0.8%) seem to be struggling the most in light of changes in consumer trends.

A study of the domestic market as a lever on turnover, highlights the growth trend in professional channels, which have experienced an increase of 1.3%, with a sell-in value of 669 million euro. Indeed, in 2016 visits to beauty and hairdressing salons picked up again, having suffered the most as a result of the economic crisis which exploded in 2008.

Influenced by a general economic recovery that is still slow, the domestic consumption of cosmetics has breathed a faint breath of life into the turnover of domestic companies, which, along with the strong levels of performance achieved by exports, has generated a significant recovery in terms of profitability. Indeed, sales abroad increased by 12.7%, accounting for 4,293 million euro. Whilst rates were somewhat slower, the trend regarding imports was also positive, growing by 9.2%, confirming the slow recovery of domestic demand, which is still focused on products offering the best price-quality ratio.

As a result of these trends, the trade balance remains largely positive, with a record value of just over 2,300 million euro.
The analysis of turnover percentages for the leading end market highlights interesting developments: mass market retailers still recorded a slight decrease, from 28.7% to 27% of the market, no longer mitigated by the incorporation of herbalist shops, which has seen a significant growth slowdown. However, export figures continue to rise, accounting for 40.9% of turnover and direct sales (6.6%).
There has been a reduction in the importance of the pharmacy sector which previously accounted for 11.5% while now it has reached 8.2%. The same scenario applies to perfume shops: their share has dropped from 12.4% to 11% although they remain an industry mainstay. In the face of an economic landscape that remains uncertain, sales trends reflect the health and competitiveness of the industrial cosmetic system and the effectiveness of the strategy which sees ongoing efforts in the field of research and innovation on the part of companies within the industry, who have been making industrial investments that are well above average.
It is perhaps useful to recall that on the occasion of Cosmoprof Bologna 2017, the Statistics Department launched an analysis which has been extended to cover the cosmetics supply chain, with a view to proposing, with the necessary adjustments, an extended measurement framework which offers a dynamic and rational overview of the phenomena which affect the chain at various levels, upstream and downstream. From cosmetic ingredients to production machinery, packaging and the finished product, the intention is to provide an overview of the "long" supply chain of the Italian cosmetics industry.
The value of the total industry turnover exceeds 15,000 million euro, with a positive trend in 2016, showing a recovery of more than five percentage points, which has been further reinforced by forecasts for 2017. The year is expected to close positively, with a growth of more than 4%.
From raw materials, which recorded a turnover of approximately 950 million euro, to machinery, with over 300 million and packaging, which has reached the figure of 3,300 million euro, the cosmetics supply chain demonstrates various distinctive characteristics specific to the sector. The upstream supply chain, for example, clearly leans towards exports, and in the case of machinery, the export-production ratio even reaches 74%, while ample room for improvement is expected for the finished product segment. Constant investment in research and innovation is common to all "links" within the supply chain, as is the difficulty of predicting scheduled, ongoing orders, a sign of prudence and uncertainty which continues to affect markets.
Thus the Italian cosmetics industry's "long" supply chain demonstrates unique dynamics, thanks to manufacturers both upstream and downstream, and companies in direct contact with the customer - all of which serve to reiterate the competitiveness and excellence of "Made in Italy" cosmetics. These companies, more than those in other sectors, have responded quickly, implementing - among other things - adequate internationalisation activities and strategies, with significant positive trends in exports.
The Italian cosmetics industry is recognised worldwide for its innovative formulations and the dynamic service of its manufacturers; added to this is the continuous study of consumer trends, which is essential for establishing itself in the counselling field. These characteristic elements have enabled the Italian cosmetics industry to make a name for itself on both traditional and emerging markets.

TABLE I

Turnover of the cosmetics industry by distribution channel (Values in millions of euro)

	2015		2016		Forecast 2017
	Value	Var. %	Value	Var. %	Var. %
Turnover Italian market	**6,164**	**1.5**	**6,208**	**0.7**	**1.2**
Total - traditional channels:	**5,503**	**2.0**	**5,539**	**0.7**	**1.2**
Pharmacies	859	1.5	858	-0.1	0.5
Perfume shops	1,148	0.9	1,158	0.9	-
Mass market and other channels (*)	2,855	1.3	2,832	-0.8	0.7
Door-to-door and mail order sales	641	8.1	691	7.8	8.5
Total - professional channels:	**661**	**-2.4**	**669**	**1.3**	**1.9**
Beauticians	172	-3.2	176	2.1	2.3
Hairdressing	488	-2.2	493	1.0	1.8
Exports	**3,807**	**14.3**	**4,293**	**12.7**	**9.5**
Global turnover of the cosmetics industry	**9,971**	**6.0**	**10,502**	**5.3**	**4.6**

(*) includes Herbalist and Single-Brand stores

Source: Statistics Departments of Cosmetica Italia

Distribution Channels and Consumption

The value of cosmetics consumed in Italy in 2016 reached the 9,900 million euro mark, a 0.5% increase on the previous year. This is a very modest growth rate but nevertheless important for the stability of figures, considering the effects that the global crisis in the autumn of 2008 has continued to have on purchasing propensity, with a significant influence on Italian consumers in the subsequent period.

The consumption of cosmetics in Italy has long been considered essential, to the point that the negative economic situation of recent years has had a marginal effect on purchases in terms of volume, but it has certainly not affected quantities. The shift in consumption choices towards opposite ends of the price spectrum, the so-called "hourglass effect", leads to a weakening in the mid-price range, generating growth in the number of items sold while value trends have slowed significantly - they are definitely growing but still at a very slow rate. A reduction in the negative effects of the economic crisis on purchasing propensity has led to a review of choices within traditional channels, while a recovery is being witnessed in the use of professional channels.

A decline - albeit slight - is seen in all the traditional channels, especially in the pharmacy and mass market sectors, while herbalist stores and perfume shops remain strong and positive trends in direct sales continue. Within the last category, online sales are still very much the major phenomenon of recent years, with strong growth (+35%) to the tune of around 230 million euro at the end of 2016.

Given the difficulty in collecting data for online sales, the statistical basis is currently being reviewed and revised upwards. Despite the slowdown in door-to-door sales, direct sales as a

whole have enjoyed above-average growth, thanks largely to the growth of the e-commerce dynamics, both as a new sales channel and as a means of strengthening distribution.
Although calculated as part of the mass market and single-brand aggregate, consumption in the herbalist channel has grown by more than one percentage point, preceded by direct sales at 21% - the best performance ever seen in this category.
The decline in figures for perfume shops seems to have halted, and this channel appears to be clawing back consumption values, curbing the involution and the downsizing of the channel, despite the transformations in selective distribution methods. These transformations are generating a growing divide: on the one hand this bears witness to the reduction in numbers and weight of consumption in traditional perfume shops, while on the other, the prominence of specialised chains is confirmed, as these are becoming more attentive to customer service and the new requirements of consumers.
Furthermore, in 2016, growth is seen in the absolute value of professional channels: consumption in hairdressing and beauty salons has increased by 1% and 2.1% respectively.
In 2016, prices remained substantially stable; this stability is linked to uneven recovery trends; of all the various channels, perfume shops have seen the most pronounced change (+7%), due to reshaping and new products, while the others are substantially holding firm, with the exception of the mass market where supermarkets and hypermarkets are which have recording significant price increases. Marginality manifests itself once again in the professional channels.
2016 saw the evolution of new channels and the radical domestic transformation of traditional sales channels, affected by the new consumer tendencies, with buyers seeking a balance between their financial resources and the need to take care of themselves. Having adopted new approaches to spending and new tendencies in terms of pre-purchase information seeking methods, consumers are remaining loyal to more economical price categories and channels, as well as continuing to purchase premium products, leading to the marginalisation of the mid-price range.
While it is true that the cosmetics industry has only marginally felt the crisis, having long established itself as a category which is characterised by "physiological" consumption of goods, and one which is largely indifferent to economic downturns, it is also true that buying habits have changed radically at all product and channel levels, highlighting the increasingly widespread phenomenon of cross-channel purchasing.

Italian Cosmesis in the mirror

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On the 8th of November 2017, in the Italian Parliament's "Nilde Iotti Hall" located in Rome, it has been held a round table with the title *Italy in the Mirror: expression, gestures and stories of our 50 years*. The book written for this special occasion reports the more significant and cultural events that have influenced the global way of living in Italy in the last 50 years, from 1967 to 2017 (Fig.1).

Fig. 1 *The Book cover.*

1967 has been, in fact, the year of the Italian Association of Cosmetic Industries foundation that was born as UNIPRO, and is today named *Cosmetica Italia*. By the different events of the last 50 years, facing up the reality, ten years after ten years, it has been established a bridge between different way of living and use of cosmetics, described by some old publicity images also (Fig.2).

Fig. 2 *Historical cosmetic publicity.*

The round table has seen as speakers Marcella Marletta, General Director of Medical Devices and Pharmaceutical Services of Italian Ministry of Public Health, Cesara Buonamici, vice director of television Tg5; on. Raffaello Vignali, secretary of Presidency at Chamber of Deputies of Italian Republic and Group Chairperson in the in the Productive Activities Committee; on Ivan Scalfarotto Deputy Minister at Ministry of Economic Development, Luigi Contu, Director of ANSA, the first Italian press agency, Nadio Delai, President of Ermeneia, one of the most important market research agencies, and of course Fabio Rossello, President of Cosmetica Italia, general organizer and coordinator of the meeting (Fig.3).

Fig. 3 *The round table: Dr Marletta speaking.*

All the speakers reported the great financial and welfare economics of the Italian Cosmetic Industry that, during the year 2017, registered a cosmetic family spending of about 11 billion with a yearly growth of more than 1% versus 2016. At this purpose, it is interesting to underline the performance of the exported cosmetics that, with more than €4,700 millions in value, have registered a positive increase of around 10%, reporting +52.3% for the lip products, +41.1% for the toothpastes, +21.9% for the compact powders, and +16,3% for the eye make-up.

The majority of the exported products have gone versus Germany, France, USA, and UK. It is also important to remember that, from 1967, all sort of everythings are changing out of all: market, consumers, distribution, communication channels and buying habits. However, according to the words of Fabio Rossello "while many events and business are changing, the capacity of Italian cosmetic products to leave a mark on the people lifestyle remained the same over the years".

Hence Beauty and Wellness, obtained by cosmetic treatments became an important reference point of *made in Italy* connected not only to the products' use but also to the work involvement of 73.000 Hair Dressers, 18.000 Beauty and Healthy Centers, 5.000 Perfumeries, 5.000 Herbalist shops, and 21.000 Pharmacies.

It is interesting to underline that 230,000 employers of the Italian cosmetic industries are equivalent to 226,000 employers of Fiat-Chrysler group operating worldwide! However, all the cosmetic products are formulated and produced according to the sensibility, needs, way of living, and dreams of the consumers with whom the industries are constantly connected, through their chemists, biologists, toxicologists, and medical advisors, and their multichannel structures and marketing organizations.

In any case, the consumer more aware of his needs and mindful of his rights, behaves in a considered manner and, before taking his final purchase decision, explores all the cosmetic products and services through the social media also by a peer-to-peer connection. Thus, awareness and role of the leading character of consumer continue to be of fundamental importance for the future industrial requirements.

On the one hand, it will be necessary to take care of the mounting network regarding naturalness, sustainability, effectiveness and safeness of the products. On the other hand it is important not only to satisfy the request of personalized products and services, and of more and better information managed peer-to-peer, but also to maintain a good relationality between the different points and centers of purchase necessary to give services to customers and consumers. The incoming market picture foresees, in fact, a further increase of the consumer empowerment who will be always aware and difficult to satisfy, requiring both a cosmetic industry capable to interpret its necessities, and professionals with an higher knowledge and culture fundamental to satisfy their requests of ameliorating their beauty and wellness. Thus, the need to produce cosmetics more and more effective made by the use of natural ingredients which, obtained by the last sustainable technologies, are respective of both the human body's physiology and the natural environment.

The incredible progress of the last twenty years on skin biology and cosmetic chemistry gives the possibility to obtain precious ingredients from the plant world by sophisticated technologies (Fig. 4). These compounds help the industry to produce skin-friendly and innovative cosmetic carriers and products not only characterized by a higher effectiveness and safeness, but also capable to maintain the ecological balance and biodiversity of our Planet (Fig.5).

Trying to understand the deep connection between the continuous evolution of welfare state and the necessity to maintain in the best way beauty and wellness of the body, the Italian cosmetic industry has invested and continues to constantly invest in research and development. As result, it has been possibly to overcome the economical and developmental crisis of the last ten years, increasing its marketing success in the national and international market.

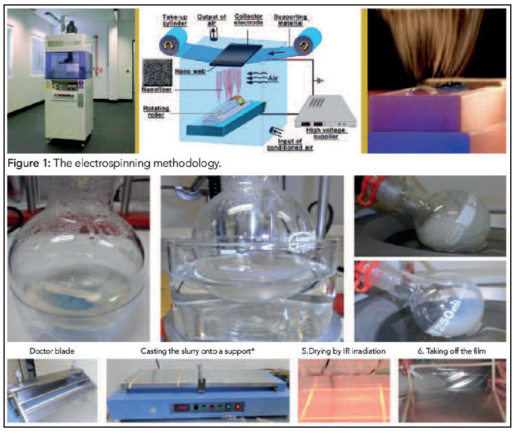

Fig. 4 *Modern lab: an example of technological equipment.*

In conclusion, all the speakers have evidenced how the Italian cosmetic industries have contributed to ameliorate beauty and wellness of all the people social classes, increasing worldwide the knowledge of the logo *made in Italy*.

This result has been possible by continuously investing in research and innovation by the help and under the umbrella of *Cosmetica Italia*.

By the words of the President Fabio Rossello this book, "reporting the historical imagines of the global world evolution during the last fifthy years with a special look to Beauty and Wellness, try to evidence the existing connections between people social, philosophical and aesthetic necessities and the scientific and technical evolution of the Italian cosmetic industries".

The ethical and technical evolutions of all the industries associated into *Cosmetica Italia* are described and illustrated by a totem that, travelling along Italy, reports and informs the consumers about the Italian Cosmetic Industry history, novelties and innovations created during its 50 years of history (Fig. 6).

Fig. 5 *The safeguarding of the Planet' Biodiversity is a must of our Society.*

Fig. 6 *Totem realized by Cosmetica Italia for the consumers knowledge.*

Chemistry and Pharmacology of Naturally Occurring Bioactive Compounds

By G. Brahmachari

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The growing interest in herbs and plants is part of the movements towards the greener economics and lifestyles. In a world where the damaging effects of food processing, over-medication, agriculture-business, increased CO_2 emissions and waste are daily more evident, plants have become one of the totem of our times. They, in fact, not only produce the bulk of the world's oxygen, but actively regulate the amount of carbon, water and hydrogen in the atmosphere, thus probably controlling the temperature range of our planet also. However, plants are not only a crucial source of energy and chemicals, but also a model of frugal use of resources and of energy conservation.

Nature is, in fact, the true inventor of combinatorial chemistry that generates molecules with highly sophisticated and unique structures. Plant's cellular machinery, in fact, is in charge of the generation of the molecular architecture constituting the basis for science and food, pharmaceutical, agricultural, and cosmetic industries' technology development. However, determining the active principle of the blends obtained from vegetables, gives place to the performance of organic and analytical chemistry.

This book, developed by **22 chapters**, reports the current discoveries and trends on the chemistry and pharmacology of the naturally occurring bioactive compounds, focusing on their chemical structures, biological activities and applications. While **chapter 1** introduces all the topics treated in the book, the solid-supported cyclization-elimination strategies to develop new categories of molecules and drugs nature-inspired is the topic focused on **chapter 2**.

Thus, it has been shown that the simultaneous cyclization and release of a product obtained from a solid support with adequate purity, is the major advantage of this method. The cycle-elimination strategy, in fact, can minimize the chemical and tethering implications by releasing the intact desired target molecules in the final step of a reaction, without consuming extra time. Beta-lactams are four-membered ring compounds widely used as useful therapeutic agents against various ailments. The Beta-lactam nucleus is, in fact, necessary for the biological activity of many antibiotics and for this reason, its synthesis had and has significant attention by chemists to establish new methodologies of synthesis.

Chapter 3 addresses the different and most significant possibility recovered to synthesize beta-lactams, amply discussing on their medicinal activities. The applications of Isatin Chemistry in organic synthesis and Medicinal chemistry is the topic of **chapter 4**, where this natural product found in the plants of genus *Isatin* and *Couropita giuanancus aubl* is focused. In this chapter, the particular Isatin

Chemistry, developed from 2000 and 2010 is discussed, reporting the many different methodologies developed up today.
Organic carbamates are the stable class of compounds derived from the unstable carbamic acid by the substitution of aminoacids end through various kinds of structurally diverse alkyl/aryl and other substitute's groups. Functionalization of amines as carbamates offers an attractive method for the generation of derivatives, which may have interesting medicinal and biological properties.
Recently, it has been shown by different researchers that the introduction of carbamate functionality significantly increases their biological activities, and many of these derivatives have been approved as drugs or are in various phases of clinical trials of products with anti cancer activity also. This is the topic of **chapter 5**. The necessity to reduce the society's dependence on imported crude oils has directed researchers' attention to the use of vegetable biomass not only as a source of energy but also as fine chemicals, such as the essential oils rich in phenolic compounds.
Chapter 6 reviews the utilization of the synthetic potential of phenolic and terpene constituents extracted from some tropical plants toward their conversion in new functionalized heterocyclic compounds. Chemical diversity of phenolics and terpenes' functionalities, in fact, allows the generation of a novel structural and skeletal diversity that are present in numerous natural products. Moreover, some aromatic tropical, plants grow fast and give a large fraction of essential oils. Additionally, the advantage of working with essential oils distilled into a single process offers today the possibility to obtain valuable constituents quickly. However, the generation of new libraries of heterocyclic compounds, recovered in the essential oils also, can significantly contribute to the search of promising models for pharmacological studies and to identify potential drugs effective against parasites, fungal pathogens, and cancer among others.
However, more than 10,000 compounds have already been discovered from the marine environment. On **chapter 7** synthesis, structure-affinity relationship and the biological activity of two azole marine products, largazole and neopeltolide, are reported and discussed. While largazole is particularly active against several colon cancer cells, neopeltolide exhibits potent inhibition of *in vitr*o tumour cell proliferation at nanometer level and also inhibits the growth of the fungal pathogen *Candida albicans*. **Chapter 8** is totally focused on omega-3 polyunsaturated fatty acids (PUFA), characterized for having double bound in the position 3, closed to the methyl terminus of the acyl chain. A fatty acid is made up of a hydrocarbon chain, from which the properties of lipid solubility derive, and a terminal carboxyl group, gives the acidic properties.
Fatty acids with chain lengths from 2 to over 30 carbon atoms are known, but the commonest are in the range of 12 to 22 carbon atoms. When each of the carbon atoms in the chain, except the two terminal ones, is bonded to two hydrogen atoms, the acids are said to be saturated, since all the bonding capacity of the carbons is saturated with hydrogen. When each of two adjacent carbon atoms is bonded to only one hydrogen, there is an ethylenediamine double bond between the pair of carbons and the fatty acid is said to be unsaturated. If the chain contains only one double bond, it is monounsaturated fatty acid and if the chain contains more than one double bond, it is a polyunsaturated fatty acid. The simplest omega-3 fatty acid is alpha-linolenic acid, that can be further desaturated by the enzyme delta-5-desaturated to yield eicosapentaenoic acid (EPA). Moreover, adding two carbons to EPA, decosapentaenoic acid (DHA) is formed. Both EPA and DHA, contained at high level in seafood and especially in oily fish, are considered to play an important role in achieving optimal health and protection against diseases.

Chapter 9 deals with the structure and biological activity of the melanins, focusing the attention on their photo protective, antioxidant, and immunomodulatory activities in humans and other mammals. The biosynthesis of these organic polymeric pigments takes place in cell, known as melanocytes, containing the bifunctional enzyme tyrosinase which catalyzes the initial events of the melanogenesis, namely the hydroxylaction of tyrosine to dopa and its oxidation to dopaquinone. Traditionally, the pigment melanins can be classified into brown to black eumelanins to yellow reddish-brown, sulphur-containing pheomelanins. They possess unique optical, electrical, free radical, redox properties, end the ability to bind metal ions and some organic compounds, including drugs and toxins. However, melanins could be used for endogenous and exogenous pigments and are also recommended as components of optical lenses and other special purpose glasses.

While **chapter 10** reports the last acquisitions on the plant metabolites oxyprenylated compounds evidenced for their promising biological activities as anticancer, anti-inflammatory, antimicrobial and neuroprotettive properties, where **chapter 11** is focused in the use of curcumin as potential therapeutic/preventive agent in the treatment of neurodegenerative disorders including Alzheimer's disease (AD), the most commonwealth from of dementia in elderly people. Thus the activity of anthraquinones, cinnamic acids, coumarins, quinones, xantoneses and other plant secondary metabolites are discussed showing the last data reported in literature regarding the extraction methods and the pharmacology activity of the different compounds.

On the other hand, curcumin seems effective in alleviating stress-inducend disorders, possibly by exerting neuroprotection and neuroendocrine functionalities in the central nervous system, where the α-beta peptides play a pivotal role as inducers of neuroinflammationd by the aging process. However, while minor signs of neuroinflammation can be found in the normal aging brain, the AD brain faces a much stronger activation of inflammatory systems, indicating an increasing amount of immunostimulation compounds.

The discovery of the physiological and pathophysiological roles of nitric oxide (NO) has became one of the most remarkable events in biology, being a paramagnetic molecule capable to stabilize an unpaired electron. In contrast to oxygen radicals, the half time of NO molecules reaches several seconds depending on the type of tissues and their physiological conditions. As result, they can easily penetrate through biological membranes and interact with inter cellular and extracellular structures, located relatively far from the place where these molecules are produced. NO plays a dual role: on the one hand, it diffuses into parasite cells and inhibits the key enzymes necessary to those cells, thereby destroys them exhibiting a protective effect against the parasite aggression; on the other hand NO, produced in excess amount, acts as a strong cytostatic agent, causing harm to the organism itself (oxidative stress condition).

These data are reported on **chapter 12** where a discussion on its structure-activity is reported, covering also the current status in the problem related to the search for new selective iNOS inhibitors, among plant secondary metabolites.

Chapters 13 and **14** are focused respectively on the X-ray structural behaviour of some bioactive steroids and the crystal structures of biologically potent xanthones with their derivatives necessary for a better understanding of their biological activities. All these compounds, in fact, are involved in a multiplicity of pharmacological activities, which reflect their different structures, also if further studies have to be performed at molecular level. The anticancer potential of gambogic acid, the statins activity for cholesterol control, the antifungal activity of some polyketides, the bio-pharmacolo-

gical effectiveness of rosmarinic acid, and the antioxidant activity of different phitochemicals are reported in **chapters 15-22** with a detailed discussion on the health-promoting activity of plants as bio factories of pharmaceutical, cosmeceuticals, and nutraceuticals.
This interesting book written by eminent chemists expert in the extraction and synthesis of natural bioactive ingredients, reports the relationship between the structure-activity of different compounds, focusing the attention on the state-of-the-art of their pharmaceutical effectiveness and safeness. For the accuracy of chemical activities and methodologies of production and control of the natural products reported, the book may be of high utility for specialists of the chemical and medical community who like to deepen chemistry and pharmacology of the plant ingredients as well as for students in chemistry who desire to enter in this fascinating field.

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Handbook of Intelligent Scaffolds for Tissue Engineering and Regenerative Medicine. 2nd Edition

By Gilson Khang

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Tissue engineering and regenerative medicine (TERM) has the role to manipulate the extracellular environment of cells inherently present in the body to enhance their biological potentials for tissue generation. TERM offers, therefore, an alternative technique to whole-organ and tissue transplantation for diseased, failed, or malfunctioning organs by the use of specialized scaffolds. Thus, porous matrices are generally employed as scaffolds for tissue-engineering purposes, promoting cell adhesion and proliferation by their microporous structure, which may incorporate relevant biological sequences. Naturally, to reach this goal it is necessary a properly designed scaffold architecture which, characterized for its specific micro structures with proper biochemical and physical properties, has dimensions comparable to the size of cells. Scaffolds can also be designed using naturally occurring proteins, such as collagen, gelatin, alginate, chitin etc, all of which favour and support cell adhesion and proliferation, being ECM-component molecules.

The extra cellular matrix (ECM) structure and its molecules, in fact, are essential for most cellular processes such as proliferation, differentiation, migration, and survival. Moreover, these molecules play a fundamental role in the development, homeostasis, and repair of tissues. Thus, ECM is a dynamic and multifactor network that surrounds cells, providing structural and mechanical support in all tissues. It mediates diverse biological processes that, crucial for tissue formation and function, play an important role in wound healing, activating intercellular signalling pathways also. Therefore, regenerative medicine has successfully assumed ECM molecules as promising biomaterials for tissue engineering. Naturally, any biomaterial is also evaluated for its capacity to induce the production of pro-inflammatory mediators and is tested with regard to the immune response that it may trigger.

This interesting book, organized in **X parts** and **44** chapters, covers all the subjects necessary to understand the many problems connected with the design and production of smart scaffolds, from the basic science to the industrial production and clinical applications. Tissue engineering and regenerative medicine covers, in fact, a broad range of applications, including repairing or replacing portions or whole tissues such as bone, cartilage, blood vessels, bladder, skin, muscle, nerve, etc.

Thus, TERM is emerging as a promise cure for millions patient suffering for Alzheimer's, spinal cord injury, muscular dystrophy, rheumatic arthritis, wound healing, burn, and many others diseases,

representing an alternative technique to whole-organ and tissue transplantation for diseases, failed, or malfunctioning organs.

To reconstruct a new tissue by regenerative medicine and tissue engineering is necessary to combine three key elements: 1) cells, such as for example chondrocytes, osteoblasts and stem cell; 2) biomaterials, such as collagen, gelatin, chitin, hyaluronic acid, polylactic acid, etc; 3) signalling molecules, such as growth factors, adhesines, defensins, etc, all inserted into appropriate scaffolds and necessary to promote cell signalling, adhesion, proliferation, migration, and differentiation. Therefore, the prerequisite physicochemical properties of scaffolds are to: a) support and deliver cells; b) induce, differentiate and conduit tissue growth; c) target cell-adhesion substrate; d) stimulate a cellular response; e) create a wound-healing barrier; f) be biocompatible and biodegradable; g) possess a relatively easy processability and malleability; h) be highly porous with large surface/volume; i) maintain mechanical strength and dimensional stability; l) be sterilizable and possibly; m) mimic the human body's environment, such as the natural ECM.

In summary, scaffold materials must contain the site of cellular and molecular induction and adhesion, and allow for the migration and proliferation of cell through porosity, maintaining strength, flexibility, bio stability, and biocompatibility. Tissue engineering, therefore, may be defined as an interdisciplinary field that, applying the principles of engineering and being active towards the development of biological substitutes, results useful to restore maintain, or improve tissue function. At this purpose, natural polymers like hyaluronic acid, collagen, gelatin, alginate, chitin and chitosan, that have shown to possess favourable cell-material interactions, are widely considered, for example, for bone tissue engineering and repairing of a bone defect using implanted material dated back to millennia.

However, from the physiological standpoint, one of the key issues in successful implantation is to overcome the initial immune reaction of the body just after implantation and during long-term residence within the defect zone. The chemical nature of the implant material is thus a crucial key to implant biocompatibility.

Apart from the chemical nature of the materials, hard-tissue of the bone repair requires a unique feature of strong mechanical stability unlike any other tissue in the body. Thus calcium phosphate ceramics and collagen as well as carbonate apatite and Mg-based polymer-reinforced metal scaffolds, are the natural choice for bone substitute materials, providing the right porosity, and better mechanical properties with good corrosion resistance and biocompatibility at the same time. It is to underline that porosity, pore size, pore orientation, and pore arrangement, all have strong effects on the mechanical properties, corrosion resistance, and biocompatibility of the structured polymers.

The biomaterial and manufacturing methods for scaffolds are focused in **chapter 1**, while **chapters 2-6** are entirely dedicated to the problems regarding the bone tissue formation and regeneration, that involve a complex cascade of signalling pathways triggering a range of cellular and biochemical, processes. Thus, the therapeutic approaches for tissue-engineered repair of bone defects have attempted to mimic its natural repairing process. By this method, it is possible to deliver a source of cells capable of differentiating into osteoblasts, induced by the growth and differentiation factors, or bio reservable scaffolding matrices necessary to support cellular attachment, migration, and proliferation. The reconstruction of new bone structure by resorption of the osteoblastic cells drives, in fact, the ability of these natural cells to remodel and regenerate themselves throughout the lifespan of a

person.

In conclusion, the clinical application of bone substitutes has to combine the successful interplay between the hierarchical organization of bone cells with the biological signals and biomaterials.

Hydrogels are hydrophilic polymer networks able to entrap large quantity of water or biological fluids, because of the presence of hydrophilic functional groups in the polymer chain. Smart hydrogels are sensitive network structures able to change their properties in response to environmental stimuli, such as temperature, pH, light, magnetic and electric fields, and ionic strength or enzymatic environment.

The nature of the hydrogels makes them particularly interesting to be used as biomaterials to be applied as wound dressings, biosensors, drug delivery carriers, and scaffolds for TERM. Recently, thermoresponsive materials have been combined with natural or synthetic polymers to form hydrogels with improved biocompatibility, biodegradability and biological functionality, thus extending the concept of their application possibilities. At this purpose they are of great interest in tissue engineering as injectable cell carriers. Thus the ideal system is a solution, just as a chitosan-glycerol phosphate-water system that, being a free-flowing liquid at room temperature, turns into a gel after administration into the body at 37°C.

Moreover, these hydrogels can be processed into spherical particles, membranes, fibers, injectable formulations etc, to be used for different biomedical applications. Thus, the encapsulation technology of microgels for clinical cell carrier and scaffold has made a great progress for regenerative medicine, offering minimally invasive cell delivery platform for tissue repair *in vivo*. Naturally the extent of biodegradability of the gel has to be precisely tuned, depending on the polymeric materials, composites and mixtures used to generate the microgels and produce the designed cells encapsulation. The controlled degradation of a microgels is also another important parameter to be taken in mind, as well as the engineered biomaterials used to closely mimic the biochemical and biophysical aspects of the native ECM, is, in fact, strictly connected with the different methods used to fabricate the polymeric microgels.

Following the growing interest dedicated to the nanotechnology and the use of natural ingredients, obtained from the renewable biomasses, the nano crystals of cellulose and chitin have attracted great attention owing to their biocompatibility, biodegradability and wide availability. These natural ingredients, hierarchically organized in microfibrillar arrangements, are the two most abundant polymers on earth which have shown to have a wide range of applications in TERM also.

Both nanochitin and nanocellulose are obtained as stable suspension by hydrochloric acid and sulphuric acid respectively. The first one exhibits positive charges at the nanoparticles surface, while the second exhibit negative charges, due to the anionic sulphate groups.

Apart from their nanodimension and inherent renewable resources in nature, one of the characteristics that mostly attracted the research on their use for the nanocomposite materials development is related to their extraordinary mechanical properties. These properties are outstanding for bio-derived building blocks, comparable to other reinforcement nanomaterials, such as clay nanoplatelets or carbon nanotubes. All these and other topics are focused and discussed on **chapters 7-16**.

In the field of engineering, the development of nanofibrous mats is one of the most promising approaches. Because of their high surface-area-to-volume ratio and the morphological structure similar to ECM, nanofibers became very appealing to be used as scaffold materials. They can be pro-

duced by several techniques but among them, the electrospinning seem to be the more promising ones for regenerative medicine.
This methodology makes the possibility to produce nanofibers from a viscous solution of polymers for obtaining a non-woven tissue. By this process it is possible to obtain electrospun nanofibers characterized by a higher mechanical strength by the use of organic or inorganic materials, solubilised or suspended into solvents or water. The so-called *green electrospinning* use water as solvent. The electrospinning platform consists of a syringe, a current power source connected with the syringe needle, a syringe pump to extrude polymers natural and/or synthetic, and a grounded plate to collect polymers stretched by the electrostatic repulsion between the polymer molecules.
Appropriate selection of biomaterials is the key factor in the construction of viable and clinically relevant engineered tissue for human tissue regeneration. However, the properties of the electrospun fibers can be optimized by controlling parameters like temperature and/or humidity of the spinning chamber, the horizontal motion of the spinneret and rotation of the target. In addition, customized scaffolds can be fabricated by depositing fibers on targets of different shapes to create innovative, enhanced hybrid scaffold materials.
Inorganic compounds can be incorporated on the surface of polymeric fibers in order to improve the cells adhesion. The spatial organization of electrospun fibers is another significant parameter for scaffold integrity, porosity, stability, as cell as cell behaviour. Thus, for example, it is possible of generating up to micrometer-range fibers, morphologically similar to native load-bearing collagen bundles.
This approach has been widely used to electrospin a variety of materials including biodegradable, non degradable, natural, and synthetic polymers for many biomedical applications. In any way it is to remember that, as previously reported, electrospinning is affected by different parameters. Among them, solution properties are considered as most influential parameters. A critical concentration of polymer solution, for example, needs to be exceeded in order to obtain a fibrous form of as-spun result. Below these concentration chain entanglements are insufficient to eject the continuous and stabilized jet.
In addition, the solution viscosity and surface tension are generally dependent on the concentration, thus having the same proportional correlation with the fiber diameter. However, one of the most attractive characteristics of the compounds electrospinning would be its productivity. Because the process is driven by strong electric field, the jets and fibers can be fabricated in a very rapid way, where the jet velocities were measured in the range of several meters per second.
All the parameters supporting the electrospinning technique are reported on **chapters 17-19**. Apart from the first conventional scaffold fabrication category made by the electrospinning technique, the second category used for the regenerative medicine is produced by rapid prototyping techniques, which include the 3D printing, processed by a wide number of biomaterials in a custom-made shape and with desired mechanical properties.
This 3D technology is a remarkable new invention developed for complex tissue/organs manufacturing that encompass a number of techniques including extrusion-based printing, inkjet printing, laser-induced forward transfer, electrostatic-based jetting, stereo-lithography, and laser guidance direct write, all amply reported by **chapters 20** and **21**.
As previously reported, regenerative medicine, at the interface between life science and engineering

nanotechnology, exploits the features of natural living cells compounded with designed biomaterials or desired drugs, tissues and organs.

Encompassing engineering and nanoscience, nanotechnology includes imaging, measuring, modelling, and manipulating matter at nano scale (from 1 nanometer to hundreds of nanometers). At this dimensions, nanomaterials possess unique physicochemical properties, including novel electronic, optical, magnetic, electrical, catalytic, and structural properties that are different from their bulk counterparts. However, at the nanoscopic realm, nanomaterials not only can brilliantly mimic the biological characteristics of natural tissues supporting cell proliferation as scaffolds, but can also efficiently guide the cells toward a desirable behaviour.

In any way for their potential use in medicine, they have to meet certain basic criteria such as to (a) establish interactions with cells and proteins without interfering their biofunctions, (b)maintain its physical characteristics after functionalization, (c) be nontoxic. Having all these properties, nanomaterials may play a crucial role in accommodating cells and further guiding them to differentiate into a specific tissue, during the regenerative process. Thus, their use to regenerate *mechanosensitive* tissues, such as bone, cartilage, and ligament/tendon, or electroactive tissues, such as neuron, skeletal muscle, and hearth, or *shear stress-sensitive* tissues, such as kidney, lung and vascular systems.

Given the vast diversity of tissues that have to be regenerated it is difficult to conceive the material(s) and tool that would be useful for all. Thus, many are the biomaterials in use to make the different suitable scaffolds for the growth of cells and tissues, which have to be not only biocompatible with the different tissues, but also easily biodegradable from the human and environment microbiome. The different possibility are discussed on **chapters 22-25**.

The natural ECM, composed of proteoglycans, hyaluronic acid, collagen, elastin, and many kinds of signal peptides and proteins, provides an interesting inspiration to develop novel functional materials for generating fully functional artificial tissues.

Phage display is one of the used platforms for protein engineering and peptide selection. It is based on a combinatorial process to identify specific binding peptides displayed on the surface of phage particles in a bacterial environment, appropriate for mammalian proteins also. Thus, phages have been genetically engineered to display a large number of peptide sequences, applied to the design of antibodies and other peptide therapeutics, as well as to display peptide motifs with a high affinity and specificity to various organic and inorganic materials. This the topic of **chapter 26**.

As reported previously, ECM is to be considered a dynamic and multifactorial network that surrounds cells, providing structural and mechanical support in all tissues and organs and regulating cellular functions *in vivo* by the signalling pathways. The ECM of the target tissues/organs, therefore, serves not only as a structural blueprint but also as a tool kit for tissue engineered. Thus, the necessity to realize biopolymers matrices, having similar structural characteristics to biological tissues and the possibility to degrade over time in order to provide the matrices for new tissues formation.

It is crucial, in fact, to understand how the ECM is organized and remodelled during the *in vivo* tissue development. Therefore, biomimetic scaffolds, designed by considering the fundamental aspects of the native tissue activities and functions, play important roles as a cell-instructive microenvironment, providing not only a desirable mechanical support but also cell-matrix interactions. The strategy and modality to design and produce the different materials and scaffold structures necessary for remake different tissues and organs is focused and amply reported on **chapters 27-31**.

Organ regeneration process, not jet well understood, represents a promising therapy that can greatly alleviate humans from disease. It holds, in fact, a tremendous therapeutic potential so that it seems necessary to find the right way to solve the different problems connected to the regenerative medicine, such as: (a) the contribution of the exogenous cells (i.e.staminal cells), in repairing and regenerating organs in absence of an innate intrinsic regenerative capability; (b) the factors, pathways, and cells that are involved in the organ's intrinsic regeneration; (c) the progress in engineered biopolymers and scaffolds.

Thus, the combination of polymer chemistry and cell biology will probably led to significant advances in the identification and understanding of matrices which provide cellular support, aiding cellular differentiation and tissue formation. Creation of engineered tissue requires, for example, a scaffold that serving as a cell carrier, has to provide structural support until the native tissue is formed *in vivo*. At this purpose, one of the challenges of developing tissues is imparting them a proper own vascularisation and synthesizing all the cellular components necessary for their development. An other problem is to find smart biomaterials capable to actively participate in the formation of functional tissues as well as to find the right molecules involved in stem cell trafficking.

Engineering replacement of tissues, therefore, requires comprehensive understanding of structure and function of the different native tissues, followed by the development of enabling technologies to fabricate scaffolds capable to replicate the key features of their normal cellular microenvironment. Thus, it is to remember some important points: (1) the biological crosstalk between cells and the scaffold is controlled by the material properties and its structural characteristics; (2) the porosity, pore distribution, and surface area play a major role by affecting the penetration of cells, the architecture of the produced ECM, and the final effectiveness of the regenerative process; finally (3) the nanoscale size and nanotopographical features of scaffolds dictate the cell formation and maintenance for regenerating the tissue into these structures.

On **chapters 32-36** organization, production and use of the different scaffolds for the regeneration of (a) mucosal trachea, (b) urinary bladder, (c) vascular tissue, (d) annulus fibrous tissue, and (e) corneal endothelium are respectively reported, describing and discussing the actual and future necessities for trying to solve the many problems connected with the availability of the natural biomaterials necessary to realize all the relative engineering tissues.

As reported by many chapters, the molecules and components surrounding the cells create a particular microenvironment responsible for the regulation of the phenotypic properties of the cells, determined by ECM.

In addition, other small molecules present a great potential as modulators of cellular responses such as motility, adhesion, proliferation, and differentiation by activating cellular signalling pathways. Thus the great difficulty to make by different methodologies scaffold systems capable to regulate tissue regeneration and engineering, mimicking the natural ECM.

The incorporation and controlled release of stem cells or growth factors from scaffold biomaterial (capable to up-regulate or down-regulate cellular activities such as protein synthesis) and built-up biomimetic surfaces by ECM molecules became popular strategies to accelerate, for example, tissue healing. These and other innovation methodologies are reported by further 8 chapters (from **37** to **44**) to attain its ultimate goal for reporting all the innovative engineered tissues, capable to efficiently regenerate human tissues and organs.

This interesting book may be considered a real *bible* of innovations totally dedicated to the regenerative medicine, estimated to represent a market of about US$ 500 billion. For the organization of its chapters, and the thousands news reported on the tissue engineering technologies, and used to produce intelligent scaffolds for regenerate human tissues and organs, the book has to stay into the library of the medical and economical community who wish to deeply know the last novelty realized and in progress in the fascinating field of regenerative medicine.

P. Morganti Ph.D.
Editor in Chief

Natural Antioxidants. Applications in food of Animal Origin

By R. Banerjee, AK. Verma, MW. Siddiqui

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In normal health, there is a balance between the formation of oxidizing chemical species and their effective removal by protective antioxidant. Thus oxidative stress is referred to as a condition of imbalanced pro oxidant/antioxidant equilibrium, in favour to the former. If the antioxidant defence mechanism fails, or if an increased flux of reactive oxidant from-and exogenous sources exceeds the antioxidant capacity, oxidative injury will result. An oxidative cell injury or a decreased efficiency of antioxidant defences contribute, therefore, to the development of different pathologies.
However, oxidative stress and lipid peroxidation are the main causes of a number of chronic diseases, the incidence of which can be reduced by the consumption of fruit and vegetables that contain different antioxidant compounds. The consume of the right and well preserved food, therefore, can prevent the cell oxidative damage by inhibiting the generation of reactive oxygen species (ROS) and active nitrogen species (RNS), scavenging free radicals or raising the level of endogenous antioxidant defenses. Few examples of ROS are alkoxyl radicals, peroxyl radicals, hydroxyl radical, and superoxide anion radical, while examples of RNS would be nitric oxide and nitrogen dioxide radical. Potential sources of free radicals could be ultraviolet rays, ionizing radiations, metabolic processes, inflammatory reactions, air pollution and smoking.
Therefore, the increased scientific evidence for protective anti-oxidative mechanisms of vegetables, fruits and some oils and peptides has led to a considerable growing interest of the food industry to support, maintain and possibly increase the antioxidant capacity of the different nutrient products distributed in the market.
This book, developed by **9 chapters**, reports the different strategies used to control the food oxidation processes.
Lipid oxidation is one of the important reactions in food and biological systems because of deleterious effect first of all on polyunsaturated fatty acids (PUFA) but also on other lipid substrates, protein and pigment, causing significant losses in food quality, health, and well-being. This oxidation is also one of the main factors limiting the quality and acceptability of meats and other muscle foods, especially following refrigerated and frozen storages with the primary formation of hydro peroxide compounds by the free radicals formation. PUFA, in fact, interact with oxygen by a mechanism of autoxidation that, by a reaction chain of initiation, propagation and termination, involves the production of free radicals. Initiation occurs when hydrogen, abstracted from an unsaturated fatty acid, results in a lipid-free radical, which, in turn, reacts with molecular oxygen to form a lipid pericycled

radical. Soon after, the propagation phase of oxidation occurs by lipid-lipid interactions, whereby the lipid peroxyl radical abstracts hydrogen from an adjacent molecule, resulting in a lipid hydroperoxide and a new lipid-free radical. The propagation reaction-chain continues until one of the radicals is removed, reacting with another radical or with an antioxidant.

This topic is reported on **chapter 1** where the oxidation mechanisms affecting the quality and acceptability of food are amply discussed. Antioxidants, neutralizing or inhibiting free radicals by preventive and radical scavenging modes, have dual role: shelf-Life prolongation and combating oxidative stress. Thus, to manufacture high -quality, stable food products, the most effective solution is often the addition of these compounds, either synthetic or natural, which can serve as chain breakers, by intercepting the free radical generated during various stages of oxidation or as metal chelators. However, consumers, concerning the bio-safety of synthetic antioxidants, have pushed food industry to seek natural alternatives such as ascorbic acid, tocopherols, polyphenols, and so forth.

They are natural, and have anti oxidative activity that is as good or better than synthetic antioxidants, make them particularly attractive for commercial food processors because of consumer demand for natural ingredients. The role of the different natural antioxidants useful for food preservation is focused a and discussed on **chapter 2**, where the more important natural compounds are reported, evidencing the role and mechanism of action they have to preserve meats and beverages.

Scientific advances, awareness of personal health, increasing healthcare costs, busy lifestyles, and technical advance in the meat industry have stimulated the *green* consumerism. Demands for the natural, ingredients, therefore, have forced researchers and industries to find natural alternatives to synthetic antioxidants. Food, in fact, leading to deterioration in colour, texture, shelf life, and overall acceptability, is easily vulnerable to oxidation because of their chemical constituents and processing, as well as post-processing conditions. However, due to the difficulty to extract from plants, maintain quality and stability of natural antioxidants, it is necessary to carry out more research work to enhance yield, for example, of plant phenolics, screen their active principles, and control their delivery and release in the meat and beverage systems. All these topics and consideration are focused on **chapter 3**. Oxidation in fish and fish products possess a high risk of quality, leading to rancid taste, off-flavour, and development of many different compounds which have adverse effects to human hearth. Oxidation is high in fish because of presence of omega-3 PUFA's susceptible of quick peroxidation because of their electron deficient double bonds. Thus, the oxidative phenomena, affect not only the lipid content causing off-flavour, colour deterioration and rancidity, but also lower the nutritive value of fish, its general freshness and the consumer acceptability.

Fish has and remains an important part of a healthy diet, being a fundamental source of a number of nutrients, particularly protein, vitamin D, retinol, iodine, vitamin E, selenium, and the essential long-chain PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Due to their prominent position in human diet and the beneficial effects on chronic degenerative diseases, the food industry and the health authorities have a joint interest in increasing the consumption of fish. Thus, development and application of natural products with antioxidant activities in fish products may be necessary and useful to prolong their shelf life and potential for preventing fish spoilage.

As previously reported, dietary intake of fruits and vegetables rich in phenolic, flavonoid compounds prevents excessive free radical formation in the cell. Hence, utilization of these natural occurring compounds in fish and poultry products will not only increase the storage stability, but also provide adequate health promoting effect to the consumer.

Poultry products are, in fact, rich of bioactive compounds which exert chemomodularity effects through a variety of physiological processes.
In conclusion, the appropriate combination of natural antioxidants can help in designing of functional foods and also tackle lipid oxidation problems. The use of plant extracts and other natural active compounds as protective antioxidants, incorporated in different food with different technologies is reported and discussed on **chapters 4** and **5**.
As amply focused previously, the addition of antioxidants is the most commonly used method of retarding lipid oxidation in fat. They increase the stability of food components especially polyunsaturated lipids, and maintain nutritional value and colour by preventing oxidative rancidity, degradation and discoloration. However, it is important to underline that any compound that is anti-oxidative under one set of conditions, can become pro-oxidative under different conditions. Thus the operative conditions are mandatory as well as type and quantity of the antioxidants used for different foods. However, the ideal antioxidant compound should have the following characteristics: (a) no harmful physiological effects; (b)absence of undesirable effects on colour, odour, or flavour; (c) effective at low concentrations; (d) compatibility with the food and ease of application; (e) survive after processing and be stable in the finished product; and of course (f) available at low cost.
In conclusion, naturally occurring antioxidant substances are at times associated with beneficial effects of foods, also if synthetic antioxidant compounds are also widely used to inhibit progress of lipid oxidation. In any way, food manufacturers have been motivated to carry out research on the use of natural antioxidants because studies have shown that such compounds are not only beneficial to the shelf life of food products but also as preventive medicine.
The safeness of the different natural antioxidant compounds, their use in the different foods according to their anti oxidative capacity, the processing efficiency, the application for processed meat, the relationship between sensory and analytical data, the extraction methods, the more in use analytical methods together with the regulatory aspects are all focused on **chapters 6-9**.
This interesting book, focusing all the aspects of the natural antioxidants from the productive methodologies of extraction, to the application techniques and the regulatory aspects for their use and the rich scientific references reported, represents an important source of news for the chemical and medical community and the food industry experts who wish to have an up-to-date on all the studies and researches carried out on the field of the natural antioxidants used as food preserving compounds.

P. Morganti Ph.D.
Editor-in-Chief

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In copertina / Front cover
complesso Nanofibrille di Chitina –Lignina.
Foto al microscopio elettronico a scansione – Su gentile concessione del CNIS, Centro Ricerca per le Nanotecnologie applicate all'Ingegneria, Univerisità La Sapienza, Roma – Italia.

Chitin Nanofibrils –Lignin complex.
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