Comparative Medicine
Copyright 2011
by the American Association for Laboratory Animal Science

Vol 61, No 6
December 2011
Pages 553–564

Abstracts of Scientific Papers

2011 Italian Association for Laboratory Animal Science (AISAL) Symposium
Biomaterials in Medicine and Animal Models
Naples, Italy • September 22–23, 2011

Main Lectures

Polymer-Based Biomaterials for Tissue Regeneration

L Ambrosio*

Institute of Composite and Biomedical Materials, National Research Council of Italy, Naples, Italy
*Corresponding author. Email: ambrosio@unina.it

The main issues related to polymer-based structures to be used for tissue repair and regeneration are the technological and scientific concerns with the design and preparation. In this context, one of the major challenges consists in the development of well-organized structures with controlled properties to obtain the best performance of prostheses and/or substrates for tissue healing and regeneration processes. Scaffolds endowed with molecular cues along with a controlled degradation profile should contribute to cell proliferation and differentiation, controlled vascularization, promotion of the remodeling of new tissue through a gradual transmission of biochemical and biophysical signals as performed by the extracellular matrix (ECM). Many synthetic, natural semisynthetic organic, and inorganic materials have been used, and even if they possess appropriate biologic and biodegradable properties, their structural performances are not completely adequate. In order to satisfy all the complex requirements, polymer gels and composites can be implemented to design an appropriate scaffold. Bioactive scaffolds, as mineralized ECM analogue for bone regeneration, with appropriate porosity and high pores interconnectivity were developed by using poly(ε-caprolactone) reinforced with calcium phosphates particles and PLA fibers. Hyaluronic acid esters in gel form are designed for nucleus regeneration in IVD pathology. Reinforcing them with degradable fibers by composite technology, phase inversion, and salt leaching technique are used as scaffolds for meniscus regeneration. Imaging and rapid prototyping technologies are implemented to design a "custom made" meniscus scaffold. In vivo results demonstrated the potential to regenerate different tissues by using an appropriate functional scaffold, although several phenomena still need to be investigated.

Life-Sustaining Polymeric Material

E Bonavoglia*, C Scalera, P Neuenschwander

Abmedica Spa, Milan, Italy
*Corresponding author. Email: bonavoglia.eliana@abmedica.it

Tissue engineering is an interdisciplinary field using a combination of engineering and life sciences to create biologic substitutes that improve, repair, or maintain biologic tissues (for example, bone, cartilage, blood vessels, and bladder). Cells are usually isolate and/or immortalize from a native source, expand in vitro and seed onto a porous scaffold. That provides 2D or 3D support for cells. It has to have a defined shape and architecture, to be bio- and cytocompatible, provide appropriate mechanical properties and a suitable degradation rate and many more to match mechanical characteristics of the replaced tissue. Ideally the scaffold only remains until the cells developed their own endogenous extracellular matrix and the diseased tissue has been healed. We have developed a synthetic elastic bio- and haemocompatible polymer which can be synthesized with adjustable mechanical characteristics and degradation rate in the body matching the needs of tissues to be replaced or supported. The pure polymer used as a scaffold is a new biodegradable and biocompatible polyester-urethane, which consists of two blocks of polymers that impart very different physical and mechanical properties to the final product: polyhydroxybutyrate*co*-caprolactone-diol (hard segment) and polycaprolactone-*co*-glycolide-diol (soft segment). Both are biodegradable polymers whose degradation products are nontoxic. Unlike traditional materials, this new polyester-urethane shows a broad range of elastic modulus, making it a potential new material for the regeneration of many types of biologic tissues. There is an increasing clinical need for scaffold to treat defects after operation, fracture or malignant tumors. However, up to date, available scaffold are very limited with respect to size and appropriate 3D architecture. For that reason we have developed a new family of polyester-urethane 3D-scaffold materials that had shown to meet essential requirements for 3D medical device. Foams, such as electrospun fiber fleeces or electrospun tubes were obtained with controlled properties and were applied as successful 3D scaffold in tissue engineering applications. Moreover recent results show how its implantation had no adverse effects. These finding represent a significant step towards the generation of a new suitable material for tissue engineering.

Regenerative Therapy in Veterinary Medicine: From Bench to Patient

M Del Bue[1,*], S Grolli[2]

[1]Animal Health Department and [2]Animal Production Department, Veterinary Biotechnology, Food Safety and Quality, School of Veterinary Medicine, University of Parma, Parma, Italy
*Corresponding author. Email: delbuemaurizio@libero.it

Although still an emerging field, regenerative medicine and

stem cells therapy in the last decade have known a growing interest and have been successfully applied in different fields of veterinary medicine. Regenerative therapy starts on a workbench of a scientific laboratory with the preparation of the cells and ends with their application to the damaged tissue of the patient. This means that technical skills and scientific competences from different area have to be gathered together to perform a state-of-the art application. Our aim is to review the main applications of regenerative therapy in veterinary medicine, both for sport and companion animals (horse, dog, cat). Isolation of mesenchymal stem cells (MSC) from bone marrow and adipose tissue, as well as their in vitro expansion and characterization will be discussed. Furthermore their possible association with scaffolds of different nature (biologic or chemical) to improve their delivery and integration into the healing tissue will be described. A further topic will be the use of allogeneic versus autologous cells: MSC exhibit an antiinflammatory and immunomodulatory phenotype, at least in vitro, thus making these cells a potential tool to induce tissue repair after allogeneic grafting. Allogeneic cells could be banked and used at the proper time, depending on the features of each single clinical case. Finally it will be emphasize how veterinary applications of regenerative medicine could be a valid preclinical model to evaluate both toxicity and therapeutic efficacy before their use in human medicine. Our clinical experience has been developed around the application of MSC associated to platelet derivative (platelet gel, platelet rich plasma) in skeletal tissue repair (tendonitis, joint diseases, and bone defects) and soft tissues wound healing. Some selected clinical cases will be described, focusing on the pros and cons of this therapeutic approach. Scientific literature and infield experiences show that regenerative medicine and cell therapy can be considered a valuable alternative to conventional therapy in many pathologies. Nevertheless, the real role of MSC and the molecular and cellular pathways they activate to improve tissue healing is far from being clearly elucidated. Furthermore, most of the available results are clinical outcomes rather than scientific evidence, and need to be carefully evaluated before we draw any conclusions about the effectiveness of these approaches.

Biotechnology and Research on Animal Models: Equal Consideration and Human Priority

K Dierickx*

Biomedical Ethics, Faculty of Medicine, Catholic University, Leuven, Belgium
Corresponding author. Email: kris.dierickx@med.kuleuven.be

During the last 50 years, our attitude towards animals in developed countries has changed, due to the estrangement from animals, the stronger involvement towards pets, and the personification of animals in children's animation. It has become more evident that humans empathize with animals and that all kinds of human feelings and convictions are attributed to animals. These changed considerations towards animals in general are not without consequences for experiments with animals and for the use of animal models in medicine. Starting from the 'laboratory animal paradox' we explore 2 fundamental ethical perspectives on the place of animals in our society: anthropocentrism and pathocentrism. We then go deeper in on different moral appraisals that follow from these positions. Furthermore, we analyze how these positions have been translated in legal normative frameworks and in the new European Directive 2010/63/EU in particular. This new European Directive will replace the old Directives in January 2012, and it acknowledges the intrinsic value of animals and states that in principle the use of live animals should be replaced by other methods not entailing the use of live animals. The Directive also states that the national perception regarding our attitudes towards animals may influence legal initiatives. We will focus on these perceptions in several European countries, especially for the use of live animal models in biotechnology, and regenerative medicine in particular. In our conclusion we will bring our main ethical findings together and we will emphasize the mutual contributions of both animal models and regenerative medicine.

Preclinical Models to Evaluate Biomaterials for Orthopaedic Applications

R Giardino*, N Nicoli Aldini, M Fini

Preclinical and Surgical Studies Laboratory and RIT–BITTA Laboratory, Rizzoli Orthopedic Institute Bologna, Italy
Corresponding author. Email: roberto.giardino@ior.it

Preclinical models are laboratory or animal studies done to determine if a new treatment is safe and effective enough to be studied in humans. They may be developed both in vitro (cell cultures) and in vivo. In vitro testing does not exclude in vivo testing; however, it provides necessary and useful results to be added to those found in the in vivo testing. In vitro methods strongly decrease the number of animals used. Only by adopting both in vitro and in vivo methods will it be possible to reduce limits and increase benefits of each single approach. In orthopedics besides new biomaterials to implement more reliable fixation devices, implants and prosthesis, great interest is represented by scaffolds to be used in tissue engineering techniques to treat large tissue defects. Before the clinical application, a material or device must be shown to be biocompatible and suitable for that application. Biocompatibility can be defined as the ability of a material to perform with an appropriate host response in a specific application. In vitro and in vivo biocompatibility tests must performed according to requirements of the International Organization for Standardization (ISO), a worldwide federation of national standardization bodies for the evaluation of medical devices. In vivo studies must be carried out according to the ethical guidelines and to the regulations on animal experimentation. The ideal animal model should mimic the anatomy, biomechanics, cell biology, and pathologic changes seen in the human skeleton and at the same time, it should be reproducible, meet appropriate ethical standards, and be economic and efficient. Most experimental preclinical studies on bone implants are performed on normal cells and healthy animals, but clinically the majority of prosthesis and devices are implanted in patients with bones structurally and functionally different from normal. Therefore, there is the requirement for preclinical models useful to reproduce bone diseases as osteoporosis. In our laboratory these models are obtained in small size (rat) and large size (sheep) animals, by performing ovariectomy to induce a postmenopausal-like osteopenic status, and then implanting the samples of the material to be tested in bone.

The ideal material should have the same osteointegration when implanted in healthy and pathologic bone. In conclusion, a reliable animal model is still indispensable for the demonstration of the benefits of any new technology and therapy with comparative studies, also by means of translational researches that allows us a rapid, bidirectional exchange of information between the laboratory and the clinical side.

From Mice to Men: The Relevance of Animal Models for Bone Research

A Ignatius*

Institute of Orthopedic Research and Biomechanics, Center of Musculoskeletal Research, University of Ulm, Ulm, Germany
Corresponding author. Email: anita.ignatius@uni-ulm.de

Animal models are essential in many fields of osteological and orthopedic research and have been crucial for acquiring knowledge about bone formation during development and healing, bone metabolism and remodeling, bone diseases and the interaction of bone with biomaterials as well as tissue engineering research. The choice of an unsuitable animal model can lead to misleading results. Therefore, animal models have to be carefully selected to match the hypothesis being tested and to ensure as much as possible analogy with humans. Many aspects, among them animal size, anatomy, physiology, age, live span, and the behavior of animals, have to be carefully considered. We must also consider availability, requirements of housing, feeding, and handling because all these factors could influence the experimental setup and the outcome of the study. Small laboratory animals such as mice, rats, and rabbits are widely used due to the good availability of inbreed strains reducing variability of the results and due to easy housing and handling. For these species a lot of techniques and tools (for example, antibodies and primers) are commercially available to analyze effects on a cellular and molecular level. However, one has to keep in mind that different inbred strains can significantly differ in bone phenotype and healing characteristics. Genetically modified mice provide a very interesting tool for bone and biomaterials research. By deletion, overexpression or ectopic expression of a gene of interest its physiologic or pathologic role or its pharmacological effect can be investigated. Additionally, immunodeficient mice or rats are often used especially in tissue engineering approaches because they allow for the investigation of human cells. Due to their small size, mice and rats are mainly used to answer basic research questions in osteology and bone healing. There are limitations in testing biomaterials because orthotopic implantation is restricted to very small implants and functional implant testing cannot be performed at all. Furthermore, biomechanical aspects are difficult to address. In addition the fast bone turnover and high capacity of bone regeneration in these animals differ extremely from humans. Larger animals such as dogs, pigs, and sheep might be more suitable. They provide the possibility to test final orthopedic devices and to consider biomechanical aspects, which are extremely important in bone remodeling, healing, and the interaction of bone with biomaterials. Furthermore, in large animals, bone structure, turnover, and healing capacity is more similar to humans. On the other hand experiments are much more laborious and expensive and the analytical tools are limited. Transgenic or immunodeficient models are not available. The talk will address the characteristics of different animal models especially with respect to bone structure and metabolism and will provide examples for animal models addressing specific questions in bone and biomaterials research.

Animal Welfare Requirements for Biomedical Research

L Martini*, M Fini

Laboratory of Preclinical and Surgical Studies, Rizzoli Orthopedic Institute–IRCCS, Bologna, Italy
Corresponding author. Email: lucia.martini@ior.it

Biomedical research requires the use of animal models to test biomaterials prior to their clinical application. The use of an animal model in an experimental test must be considered only according to the 3Rs statement and considering the benefits in terms of animal welfare and quality of science. The resulting societal benefits from the application of these concepts require an action not only at local and national level, but most importantly at international level. The International Organization for Standardization (ISO) produced in the 10993 series concerning the biocompatibility of medical device and materials contains a specific part on International Standard on animal welfare: the ISO 10993-2 part 2 animal welfare requirement; this ISO standard specifies minimum requirements for the use of animal in biologic testing; the first edition of the ISO 10992 part 2 was published in 1992 on the basis of the 3Rs statement and EC/609/CE, with a fully revised second edition published in July 2006; while the first edition focused on general concepts and a few essential requirements, the revision was written under the premise that to promote scientific validity it is necessary to ensure animal welfare throughout the study. The key provision of ISO Standard 10993 part 2 is to promote good science to minimize the use of animals and promote nonanimal tests. When animals are used in a medical device evaluation, the study must be conducted humanely according to ethical and scientific statements: the application of high standards of animal care during all tests and a specific declaration concerning the minimization of pain and suffering and distress and competent personnel. The ISO statement requires specific aspects on: study design, animal species, breed, and numbers that must be employed, sources of animal and specific health status description, with the statement of humane endpoint of the study and description of euthanasia methods. In the strict application of the 3Rs concept, the ISO 10993-2 also considers the reuse of animals. A statement of all of these aspects promotes a scientific validity in a study's design in biomedical research to avoid unnecessary use and pain of animals. The assessment of pain and stress inflicted on experimental animals not only responds to an ethical need, specific legal requirements, and international standard but can also serve to evaluate the experiment in terms of cost–benefit, where the cost is represented by pain and stress imposed on the animals and the benefit is expressed in terms of scientific progress that is believed to be achieved by the particular experiment. The veterinarian must balance his role between scientific research and care of animals and ensure, with his cooperation with all research staff, a correct and scrupulous evaluation of the degree of pain and stress inflicted on experimental animals and animal welfare that are part of every experiment employing in vivo models.

Cardiovascular Regenerative Medicine in a Small-Animal Model of Acute Myocardial Infarction

C Ventura*

Laboratory of Molecular Biology and Stem Cell Engineering, National Institute of Biostructures and Biosystems, and Cardiovascular Department, S Orsola, Malpighi Hospital, University of Bologna, Bologna, Italy
Corresponding author. Email: carlo.ventura@unibo.it

While stem cells hold promise for cardiovascular repair, multiple obstacles, including poor cell viability and delivery efficiency, uncertain fate in vivo, and the need for ex vivo expansion, still remain on the route to clinical application. In this study, we aimed at developing an effective strategy to clear the hostile environment of a damaged heart and achieve effective cardiac "reverse remodeling" prior to stem cell transplantation. A direct injection of a hyaluronan mixed ester of butyric and retinoic acids (HBR) into infarcted rat hearts afforded substantial cardiovascular repair and recovery of myocardial performance, as supported by immunohistochemistry, Magnetic Resonance and Positron Emission Tomography (mPET) Imaging. HBR restored cardiac ^{18}F-FDG uptake, increased capillary density, lowered the number of apoptotic cardiomyocytes, and led to the recruitment of endogenous Stro-1-positive stem cells. In isolated rat cardiomyocytes and Stro-1 stem cells, HBR enhanced the gene transcription of VEGF, HGF, KDR, Akt, and Pim-1, as well as the secretion of VEGF and HGF. These findings suggest the recruitment of myocardial and Stro-1-positive cells into a proangiogenic paracrine circuitry of cardiac repair. In infarcted hearts, as well as in isolated cardiomyocytes and Stro-1 cells, HBR increased histone acetylation, compared with untreated samples, suggesting that this signature of HDAC inhibition by the butyrate moiety may represent a major molecular trait for the transcriptional effect of the mixed ester. The finding that efficient cardiac rescue can be chemically afforded without stem cell transplantation may pave new perspectives in regenerative medicine. Currently, the needs of cell expansion will significantly delay stem cell transplantation compared with the onset of infarction, and concomitant scarring will contribute to the development of heart failure. Myocardial injection of HBR, affording early and sustained cardiac repair during the acute phase of a heart attack, may be the prelude for autologous stem cell transplantation to enhance long-term potential for cardiovascular cell therapy.

Premio AISAL 2010

Electrophysiologic and Behavioral Characterization of Bortezomib-Induced Painful Peripheral Neuropathy in Mice

VA Carozzi[1,*], C Renn[2], P Rhee[2], D Gallop[2], SG Dorsey[2], G Cavaletti[1]

[1]Neuroscience and Biomedical Technologies Department, University of Milan-Bicocca, Monza (MB), Italy; [2]University of Maryland Center for Pain Studies, School of Nursing, University of Maryland, Baltimore, MD
Corresponding author. Email: valentina.carozzi1@unimib.it

Bortezomib (BZ) is a potent and selective first-in-class proteasome inhibitor that is mainly used for the treatment of relapsed, refractory multiple myeloma. Peripheral neuropathy (PN) is a significant side effect of BZ-based chemotherapy and one of the major reasons for a dose reduction and discontinuation of life-saving therapy, but its mechanisms remain poorly understood. Metabolic changes resulting from the drug accumulation in the dorsal root ganglia (DRG) and mitochondrial dysregulation may contribute to the pathogenesis of the painful, axonal, sensory distal neuropathy. Moreover BZ-induced pain is associated with deficits of all 3 major fiber types (Aβ, Aδ, and C). We have characterized the pain sensations produced by 4 wk of intravenous administration of BZ in BALB/c mice assessing: 1) changes in mechanical/thermal thresholds through behavioral tests and 2) changes in the electrical activity of wide dynamic range neurons of the spinal dorsal horn of the spinal cord by electrophysiologic recordings. BZ induced the development of significant mechanical allodynia starting from the first week of treatment to the end of the experiment. Moreover, the electrophysiologic assessments performed in the spinal dorsal horn revealed that, despite the incapacity of the drug to cross the blood-brain barrier, it resulted in an increase in the activity of the wide dynamic range neurons, particularly after light stimulations of the hind paw. Our results demonstrate that the chronic treatment with BZ produces a painful neuropathy in a mouse model. Therefore, this model will enable us to conduct further mechanistic studies of BZ-related antineoplastic activity, peripheral neurotoxicity and pain and can be used as a reference in the preclinical discovery of new neuroprotective as well as of analgesic compounds.

Oral Presentations

Cnidarian Models for Nanotoxicology

A Ambrosone *, L Mattera, V Marchesano, A Tino, C Tortiglione

Istituto di Cibernetica E Caianiello, Consiglio Nazionale delle Ricerche, Pozzuoli, Italy
Corresponding author. Email: a.ambrosone@cib.na.cnr.it

In the emerging area of nanotechnology a key issue is related to the potential impacts of the novel nanomaterials on the environment and human health so that this technology can be used with minimal risk. Specifically designed to combine on a single structure multipurpose tags and properties, smart nanomaterials need a comprehensive characterization of both chemicophysical properties and adequate toxicological evaluation, which is a challenging endeavor: the in vitro toxicity assays that are often employed for nanotoxicity assessments do not accurately predict in vivo response. To overcome these limitations and to evaluate toxicity characteristics of cadmium telluride quantum dots in relation to surface coatings, we have employed the polyp *Hydra* as model system. *Hydra vulgaris* (Cnidaria, Hydrozoa), is a dipoblastic animal, at the base of the metazoan evolution, composed of just 2 epithelial cell layers (an inner endoderm and an outer ectoderm facing the low ionic strength medium) with few interspersed specialized cell types, a neuronal net controlling functions and physiology. This structural complexity, simpler than vertebrates, with central nervous system and specialized organs, but much complex compared with cultured cells, makes *Hydra*

comparable to a living tissue which cells and distant regions are physiologically connected. Herein, by testing CdTe nanocrystals (QD) of different surface coatings, we investigated the impact of QD on *Hydra* physiology and cell biology. In particular QD uptake, accumulation, cellular and molecular effects were monitored in vivo, at whole animal level. We assessed acute and sublethal toxicity by scoring for alteration of morphologic traits, population growth rates, and influence on the regenerative capabilities of *Hydra*. Moreover, in order to evaluate the cytologic effects, we measured the cellular proliferation rate in presence of NC. Looking for the induction of signal transduction pathways, gene expression of key genes of cell cycle, apoptosis and oxidative response were also examined after short- and long-term NC incubation. Thus by using different approaches spanning from animal biology to cell and molecular biology we provide an exhaustive biologic evaluation of CdTe nanocrystals, discussing their toxicological effects for living organisms.

Osseointegration of Resorbable Cement Containing the Radiopaque Agent $BaSO_4$ after Long-Term Implantation

R Bindl[1,*], S Schorlemmer[2], P Augat[2], A Ignatius[1]

[1]Institute of Orthopedic Research and Biomechanics, Centre of Musculoskeletal Research, University of Ulm, Germany; [2]Traumacenter Murnau, Murnau, Germany
[*]*Corresponding author. Email: Ronny.Bindl@uni-ulm.de*

Resorbable calcium phosphate cements are clinically used for bone defect treatment and augmentation of osteoporotic bone in spine surgery. To control the distribution of the cements clinically, it is important to have good visualization of the materials under X-ray. To increase radiopacity $BaSO_4$ could be added. However, the biocompatibility of $BaSO_4$ is not yet clear. Our goal was, therefore, to analyze the influence of $BaSO_4$ on the osseointegration and biocompatibility of calcium phosphate cements. Two cements were used: KyphOS^R and KyphOs^R+ $BaSO_4$. The radiopaque cement contained 15% $BaSO_4$ as an additive. The cements were implanted bilaterally under partly loaded or minimally loaded conditions, into the cancellous bone of the tibia and the distal femoral condyle of 20 adult female merino sheep. The sheep were euthanized after 1 (n = 8), 2 (n = 8), and 3 (n = 4) y. Undecalcified bone histology and energy dispersive X-ray (EDX) analysis were performed. To evaluate the mechanical properties of the cements a biomechanical indentations test was performed. To determine significant differences the Wilcoxon test was used. Histologically, there were no differences between the cement with or without $BaSO_4$ in terms of the biologic reactions and the material degradation after 1, 2, and 3 y of implantation. Both types of cements were completely surrounded by a bony lamella, without any intervening connective tissue. Mono- and multinuclear cells with incorporated particles could be observed near the implant surface. The cellular reaction was similar after 1, 2, and 3 y and did not invoke pronounced inflammatory reactions or osteolysis. The stiffness and hardness of the implanted cements determined by the indentation test ranged between 670-1500 N/mm and 29-66 N/mm^2, but did not differ between the 2 types of cements and the different implantation periods. The self-setting, resorbable calcium phosphate cement KyphOs^R revealed excellent biocompatibility and osteoconductivity after long-term implantation. The addition of $BaSO_4$ did not change the cellular reaction to the cement at the implantations site. There were no signs of an increased inflammatory reaction. The degradation behavior and the mechanical properties during implantation were also not influenced. It can be concluded that the addition of $BaSO_4$ could be an option to increase the radiopacity of resorbable calcium phosphate cements.

Meeting the Challenge of Nanosafety with Animal and Cellular Models

ER Brown[*], S Piscopo, R De Santis, MR Pinto, I Zucchetti, D Mellilo

Department of Animal Physiology and Evolution, Stazione Zoologica, Anton Dohrn, Naples, Italy
[*]*Corresponding author. Email: euanbrown2@gmail.com*

Nanomaterials are becoming increasingly important in their applications and uses in many industries, including consumer products and healthcare. Engineered nanoparticles (NP) represent a major part of this growth. However, an understanding of their toxicological properties has not kept pace with the exponential rate of increase of research into their synthesis, and application. Particularly, research into their behavior, impact, and fate in aquatic environments is at a very early stage. We seek to address this problem in an integrated collaborative project on defined engineered nanoparticles where we relate their structure to function at successive levels of molecular, cellular, organismal, and ecosystem organization. First, we used as a starting point highly defined nanoparticles (SiO_2 and ZnO) of 30 and 170 nM diameter which have been fully characterized by dynamic light scattering (DLS), and electron microscopy. Subsequently these NP were tested on a panel of model membrane, cellular, and animal systems for which high quality biophysical and cellular information is already available. The panel ranged from isolated mammalian cells, native marine and terrestrial animal cells (for example, ascidian blood cells), and whole animal fertilization and embryologic developmental assays (Ascidians). The outcomes were measured by direct biophysical assay of biologic membranes and membrane proteins (voltage clamp), phagocytosis assays, and reproductive success. Finally the location of NP in cells and tissues was resolved by electron microscopy. The results show that ZnO_2 NP represents a significant hazard across the assay panel at less than 10 µg/mL whereas SiO_2 NP does not show cross-panel effects. These results are due to the nature of the fixed charges on the surface of the NP, and their effective diameter (170 nm particles have no significant effects). The endpoint of the project is to fit this data into a multicomponent model. In so doing it will fill an important knowledge gap and inform the EU's code of conduct for responsible nanosciences and nanotechnologies research, which aims at future regulation by the EU (REACH Directive) and Member states.

Pharmaco-Toxicological Assessment of Polymeric Core-Cross-linked Micelles with Gradually Releasing Paclitaxel

C Canapè[1,*], A Coppo[1], S Bussi[1], C J F Rijcken[2,3], A Maiocchi[1]

[1]BRACCO Imaging SpA, Bracco Research Centre, Colleretto Giacosa, Tuscany, Italy; [2]Department of Pharmaceutics, Faculty of Sciences, Utrecht University, Utrecht, The Netherlands; [3]Cristal

Delivery BV, Utrecht, The Netherlands
*Corresponding author. Email: Carolina.Canape@bracco.com

This work has been done in the framework of Meditrans project, an FP6 Integrated Project funded by the European Commission under the "nanotechnologies and nanosciences, knowledge-based multifunctional materials and new production processes and devices" thematic priority. Core-crosslinked micelles with gradually releasing paclitaxel (PTX), coded PTXL2 PM have been developed by UU with the aim of prolonging the exposure to PTX therapeutic levels and decreasing the toxicity of cosolvents which are used in the current Taxol formulation. Our aim was to assess the safety pharmacology and the toxicological profile of this nanomedicine, evaluating behavioral, neurologic, and autonomical responses (Irwin method) and determining the maximum tolerated dose (MTD) after single intravenous administration to CD1 mice. The PK of the formulation was investigated after single intravenous administration in CD IGS rats. Mice treated with a single dose of PTXL2 PM (20 mg/kg PTX) and with empty micelles formulation (PM) showed, during Irwin test, slight and rapidly reversed signs of excitation, indicated by the minimal increase in reactivity, irritability, vocalization, respiratory rate. The MTD was evaluated after single intravenous administration of increasing doses of PTXL2 PM: no mortality and body weight loss ≥ 20 % of control were observed up to 86 mg/kg PTX dose level. Histopathologic examination mainly revealed slight-marked vacuolation of liver Kupffer cells, slight vacuolation of spleen macrophages and slight kidney tubular cells microvacuolation, as result of pegylated nanoparticles clearance by macrophages of the mononuclear phagocyte system. In the PK study (10 mg/kg PTX) blood samples were collected at 1, 4, 24, 48, 72, and 96 h and 7 d after treatment. Results indicated at 1 h (t_{max}) a C_{max} value in plasma of 100 µg/mL for total PTX (micellar bound + free PTX), and a plasma concentration of free PTX of 20 µg/mL. Free PTX levels >1 µg/mL were still detectable up to 72 h after treatment. These data indicated that PTXL2 PM increased the systemic circulation time of PTX while in time PTX is released and can exert its therapeutic activity. In summary, this nanoparticulate formulation can provide a useful dosage form for PTX intravenous administration. We are now taking the step to further develop this highly promising platform technology into actual nanomedicines with a clear added value for patients, clinicians, and companies.

In Vivo Gelatin Microspheres Distribution and Release in Experimental Model of Myocardial Infarction

L Guarino[1], MG Monti[1,*], G Imparato[2,3], A Luciani[2,3], N Castaldo[1], V Petrillo[1], C Natale[2,3], PA Netti[2,3], A Cittadini[1]

[1]Department of Internal Medicine and Cardiovascular Sciences, [2]Interdisciplinary Research Centre on Biomaterials (CRIB) University Federico II, and [3]Center for Advanced Biomaterial for Health Care @CRIB, Naples, Italy
*Corresponding author. Email: mariagaia.monti@unina.it

Gelatin hydrogel microspheres (GHM) are nonviral vectors for gene and/or protein delivery that were utilized to obtain sustained and controlled release of various drugs targeting specific organs. GHM spherical shape seems to promote drug retention in the injured myocardium better than other preparations. The purpose of this study was to set up a delivery technique of GHM in infarcted myocardium and to validate in vivo dynamic of GHM release. Gelatin microspheres were prepared using single emulsion method and encapsulation of hVEGF or IGF1 was achieved by diffusional loading. Rats were anesthetized with zolazepam hydrochloride and tiletamine hydrochloride (20 mg/kg) and atropine (0.05 mg/kg), myocardial infarction (MI) was performed by ligation of the proximal left anterior descending coronary artery and confirmed by ST-segment elevation. Ten minutes after MI, a 100 µL of sterile suspension with 20 mg/mL GHM was delivered around the infarct area in 10 small injections. To verify the distribution of GHM, 10 rats were treated with red labeled GHM that were localized and counted in frozen sections 10 min after the treatment. To investigate about in vivo release, 40 infarcted rats were randomized to receive empty GHM or GHM embedded with IGF1 or with VEGF or with a combination of both. Human IGF1 and VEGF heart content was analyzed at 1, 7, 14, and 28 d after the surgery with Western blot and specific antibodies for human isoforms. Some hearts were also processed for histology after 1, 7, 14, and 28 d from surgery. Sections were stained with picrosirius red and GHM were localized in normal and polarized light. The microspheres were efficiently retained in the heart and homogeneously distributed in the targeted areas of injection. After 1 d microspheres appeared packed at the site of the injection, and after 7 and 14 d they were evenly distributed at the infarct borders. Some spheres were also found in the remote area (< 8%). After 28 d microspheres were not detectable. Homogenates of remote and periinfarcted zone showed a detectable amount of human IGF1 and VEGF after 1, 7, and 14 d after delivery that was significant lower after 28 d. In this experimental model, biodegradable microspheres were homogeneously distributed in the infarcted myocardium and allowed an optimal delivery locally exposing myocardial tissue for a prolonged period and in an appropriate concentration gradient.

Platelet Gel Preparation Technique and Its Use in Tissue Repair Processes in Dogs: A Pilot Study

A Pelagalli[1,*], V Drago[2] M Izzo[2], B Lamagna[2], L Avallone[1], F Lamagna[2]

[1]Department of Biological Structures, Functions, and Technologies; [2]Department of Clinical Sciences, School of Veterinary Medicine, University Federico II, Naples, Italy
*Corresponding author. Email: alpelaga@unina.it

Platelet gel (PG) is a substance that is created by pheresing platelet-rich plasma (PRP) from whole blood and combining it with thrombin and calcium or other activators to form a coagulum. This coagulum or "platelet gel" has an extremely wide range of clinical healing uses from dental surgery to orthopaedics and plastic surgery in human. Also in veterinary medicine in the last years platelet gel has been prepared in order to speed up and improve the process of regeneration of mesodermal tissues through the release of important growth factors. The literature reports about this report success of platelet gel application in horses. Differently very poor is the literature on canine platelet gel. The purpose of this study was, therefore, to evaluate the efficacy of platelet gel preparation technique and its use in tissue repair pro-

cesses in dogs. The study was carried on 9 clinical cases of dogs received at our institution. A baseline hematocrit and platelet count was obtained from each in order to exclude coagulation deficit. Then 20 mL of whole blood was drawn from each dog and drained into a sterile citrated tube. Half of the total blood volume was used for the preparation of autologous thrombin. Blood (10 mL) was centrifuged at 180, 800, and 1500 rpm in order to obtain platelet concentrate. Then, a sterile plastic dish was coated with the platelets and the activator could be added to the PRP after placing it in the dish. The gel obtained was then placed over the wound in the different clinic cases. The dogs were checked 15 to 20 d after surgery with different results. In many cases the response was optimal. The main difficulties are given the actual root of the gel, which is made difficult by the absence of mechanical restraint by the tissues overlying. The effectiveness of platelet gel was highlighted particularly in the early stages of the healing process, allowing the activation of coagulation phenomena in a more rapid. In conclusion, it is possible to say that the angiogenic action of platelet gel in promoting nonspecific cellular response would allow its use to facilitate and accelerate a process that also uses other sources of supply (stem cells and others).

Effect on Proliferation and Osteogenic Differentiation of hMSC by Sol-Gel Composite Scaffolds

MG Raucci*, V D'Antò, V Guarino, L Ambrosio

Institute of Composite and Biomedical Materials–CNR, Naples, Italy
*Corresponding author. Email: mariagrazia.raucci@imcb.cnr.it

Tissue engineering represents a new field that aims to grow complex, 3D tissues or organs to replace damaged tissues. In craniofacial field, the tissue engineering approach relies on the principle that mesenchymal stem cells (MSC) are virtually capable of generating all craniofacial structures. Cells with characteristics of adult stem cells have been isolated from bone marrow (BMSC) the dental pulp (DPSC), and the periodontium (PDLSC), and they all have an osteogenic potential. Organic-inorganic hybrid materials obtained by sol-gel method might provide promising scaffolds to support bone regeneration. The aim of this research was to develop and to evaluate the osteoconductive properties of hybrid composite HA/PCL scaffolds synthesized by sol-gel method. Hydroxyapatite/Polycaprolactone (HA/PCL) composite scaffolds were prepared by sol-gel method at room temperature. Biologic analysis was executed to evaluate the bioactive potential of the hybrid composite material by the synthetic body fluids (SBF) biomimetic approach. Finally, the response of 2 different lines of human mesenchimal stem cells (hMSC) in terms of cell proliferation and differentiation into osteoblastic phenotype was evaluated by using Alamar blue assay, and alkaline phosphatase activity. The morphology of cell-scaffold constructs was analyzed by scanning electron microscopy (SEM) and histology (hematoxylin and eosin stain) after 15 and 35 d. Biologic analysis by SBF treatment shows that the presence of a bioactive component such as the nanoscale HA particles in the composite materials improve the development of an apatite coating. Moreover, cell proliferation strongly increased over culture time with a significant difference between BMSC and DPSC cell lines and between the cells growth in the basic medium and the cells growth in the osteogenic medium. Furthermore, the scaffold supports the osteoblastic differentiation of BMSC. Meanwhile, DPSC showed lower amounts of ALP, maybe due to their origin (dental pulp). At day 15, SEM micrographs show that cells migrated through the pores into nearly every corner of the scaffold and were able to adhere and to proliferate onto the wall of the pores (Figure 1). Our results indicate that tissue engineering by means of hybrid composite HA/PCL scaffolds represent a new therapeutic strategy to repair craniofacial bone defects and may have a strong impact in the treatment of patients affected by complex craniofacial defects.

Detection of Polymer-Specific Antibodies for In Vivo Biocompatibility Testing: Comparative Experience from Different Animal Models

M Schlosser[1,*], L Wilhelm[3], U Walschus[1], A Hoene[2], M Patrzyk[2], H Goldmann[4], R Zippel[5]

[1]Institute of Medical Biochemistry and Molecular Biology and [2]Department of Surgery, University of Greifswald, Greifswald, Germany; [3]Department of Surgery, Hospital Demmin, Demmin, Germany; [4]Aesculap AG and Cmpany KG, Tuttlingen, Germany; [5]Department of Surgery, Elbland Hospital Center, Riesa, Germany
*Corresponding author. Email: schlosser@uni-greifswald.de

Polymers are widely used as biomaterials. Once thought to be inert, they were found to be prone to inflammation-induced biodegradation by phagocytes and T lymphocytes. In contrast, little is known about humoral immune reactions against polymeric implants with conflicting clinical case reports regarding specific antibodies (Ab). However, these contradictory results are probably due to case-to-case heterogeneity and different assay sensitivity. Animal models could therefore help to study the phenomenon of material-specific Ab. The following polymers were examined in vivo: 1) polyurethane (PU), cellulose acetate (CA), regenerated cellulose (RC) biosensor membranes, implanted subcutaneously in rats; 2) collagen-coated polyethylene terephthalate (PET) vascular graft segments, repeatedly implanted intraperitoneally with or without complete Freund Adjuvant (CFA) application in mice and rats; 3) collagen-, gelatine- or HSA-coated PET grafts and uncoated polytetrafluoroethylene (PTFE) grafts, implanted intramuscularly in rats; 4) 3 differently constructed collagen-coated PET vascular grafts, implanted functionally in pigs; (5) 2 PET-based vascular grafts coated with gelatine or a resorbable polymer mixture (lactide, caprolactone, glycolide), implanted functionally in sheep. Serum Ab were detected by enzyme immunoassays with material particles or segments as targets. PU, CA, and RC induced Ab in rats with varying immunogenicity and considerable individual variability. The same was found for PET in mice, rats, pigs, and sheep, with different kinetics depending on species and implantation site. CFA application, simulating a bacterial infection, as well as repeated implantation boosted Ab levels. Ab kinetics was influenced by graft coating in rats, sheep, and pigs, and by graft construction in pigs. Furthermore, a possible association of anti-PET Ab with local tissue reactions was found in pigs. In contrast, no Ab were detected for PTFE or the resorbable polymer. While antigenic structures of polymers remain unknown, microparticles and leachables due to biodegradation may be immunogens.

Especially phagocytic cells are involved in inflammation and can act as antigen-presenting cells. Further studies are needed to clarify a possible clinical relevance of these Ab. However, the results indicate that the humoral immune response could be an additional parameter for in vivo biocompatibility testing and demonstrate the importance of animal studies.

In Vivo Effectiveness of a Hybrid Vascular Graft Fabricated Using a Combination of Electrospinning and Fused Deposition Modeling

C Spadaccio[1,*], A Rainer[2,4], F Nappi[6], P Sedati[3], F De Marco[5], S De Porcellinis[4], M Chello[1], M Trombetta[2]

[1]Cardiovascular Surgery Area, [2]Chemistry and Biomaterials Laboratory, and [3]Image Diagnostics Area, Center of Integrated Research, Campus Bio-Medico University, [4]Biomatica, and [5]Istituto Regina Elena, Rome, Italy; [6]Fondazione Girolamo Orlando, Pescopagano, Italy
[*]*Corresponding author. Email: c.spadaccio@unicampus.it*

To test the in vivo effectiveness of a newly developed bioactive tissue engineered vascular conduit fabricated using a combination of electrospinning and fused deposition modeling. A tubular bioactive poly(l-lactide) electrospun scaffold reinforced with a single-layer helical poly(ε-caprolactone) coil was produced by means of a computer aided tissue engineering approach, combining electrospinning and fused deposition modeling techniques. Vascular prostheses were engineered to release heparin with a sustained profile and implanted in vivo, following in vitro testing, as an aorto-aortic bypass in a rabbit model. Briefly, the infrarenal aorta was exposed through a midline abdominal incision. After partial clamping of the vessel using a sidebiting clamp, engineered grafts of 4 cm in length were sutured in an end-to-side fashion to the aorta to realize an aorto-aortic bypass. The aortic tract among the 2 anastomoses was then ligated to ensure that all blood was supplied through the graft. Grafts were explanted 14 d after implantation. Ten days before euthanasia, animals underwent CT scanning with intravenous contrast agent and volume-rendered reconstructions were obtained. Scaffolds were explanted and routinely processed for histologic analysis. Armored scaffolds showed optimal mechanical and suture retention properties. Heparin release profile showed an initial releasing burst in the first 24 h followed by a sustained release after 4 wk. Analysis of CT scans and 3D volume rendering demonstrated patency of the graft implanted with adequate distal run off. Histology following explantation showed adequate endothelialization with flattened cells lining on the luminal surface of the tubular structure. No signs of thrombosis could be detected. The outer layer of the prosthesis was populated by elongated cells resembling smooth muscle cells. We developed a tailored engineered device able to overcome the hurdles commonly hampering vascular grafts, thrombogenicity, and lack of mechanical strength. Unlike other approaches, we did not preseed scaffolds with autologous cells. On the opposite, we focused on the optimization of the biomimetic design of the scaffold and its differentiating abilities. The rationale underlying this study concerned the possibility to exploit the endogenous reparative capabilities of the body and to guide these regenerative resources toward tissue restoration by means of a tailored absorbable material.

Hydroxyapatite Suitability for Rat and Rabbit Implantation

KB Violin[1,*], TS Goia[1], DS Gouveia[1], C Ribeiro[2], JC Bressiani[1], AHA Bressiani[1]

[1]Material Science and Technology Center, IPEN, São Paulo, Brazil; [2]Center of Engineering, Engineering, Modeling, and Applied *Social Sciences* Center, UFABC, Santo André, Brazil
[*]*Corresponding author. Email: kalanviolin@gmail.com*

To accurately evaluate in vivo hydroxyapatite (HA)-based implants, it is important that they be an appropriate fit for the model. This is necessary so we can recover the most from them during specimen analyses, and it is especially true when we take into account the differences of laboratory animals' sizes and metabolism. Two types of HA-based implants were evaluated, one consisting of a macroporous HA:β-TCP (MHA) processed by direct consolidation using the protein-action technique, a globular protein based consolidation with ovalbumin, and one HA nanopowder with addition of Mg2+ 0.36%wt (NHA) synthesized by neutralization method, inside an ultrasound bath. The MHA sample shape to implant in the animal model was obtained by cutting the consolidated material with a 4-mm diameter core-drill. The NHA was used as powder. Wistar rats and New Zealand white rabbits were used for the in vivo tests. The implant surgery was performed under deep anesthesia with a pharmacologic association of 5 mg/kg xylazine and 35 mg/kg ketamine for rats and 1 mg/kg xylazine and 20 mg/kg pentobarbital for rabbits. The osseous defects were performed with a 2-mm drill in the rat's femur and 4 mm in the rabbit's tibia. After the surgery, the animals received a 0.2-mL dose of pentantibiotic as a prophylactic and a 10 mg/kg analgesic dose of morphine. Rats were evaluated after 4 wk and rabbits after 8 wk. Porous scaffolds, such as MHA, has limitations concerning the size of the sample, as its fragility increases in small sizes, making the adjustment to cut a 2-mm diameter sample very difficult and unpromising. However, a 2-mm bone defect limit in the cross section of the rat's femur is the reasonable limit to test the implants properly. The rabbit's tibia has a much wider area to perform a bone defect, but in large implant sites, particulate materials tend to collapse in bone defects greater than 2 mm. The 2-mm defect of the rat's femur was suitable to hold the powder compacted in situ. Also, its higher metabolism, which leads to half the time for the repair, compared with the rabbit's also showed some particles of HA inside the repaired bone in the process of remodeling. Furthermore, during the bone repair of MHA in rabbits, we could observe bone growth inside the pores towards the center of the implant. Due to the small size and faster metabolism of rats, they are a better fit to test implants of nanopowder, while rabbits' larger, long bones and slower metabolism are a better fit to evaluate macroporous implants.

Morphometric Analysis of Local Inflammatory Reactions after Simultaneous Implantation of Modified Titanium Implants: A Model for Comparative Evaluation of Biomaterials

U Walschus[1], A Hoene[2], M Patrzyk[2], A Kochanowski[1], B Neukirch[1], L Wilhelm[3], K Schröder[4], R Willumeit[5], HG Neumann[6], M Schlosser[1,*]

[1]Institute of Medical Biochemistry and Molecular Biology and

[2]Department of Surgery, University of Greifswald, Greifswald, Germany; [3]Department of Surgery, Hospital Demmin, Demmin, Germany; [4]Leibniz Institute for Plasma Science and Technology, Greifswald, Germany; [5]Helmholtz-Zentrum Geesthacht, Geesthacht, Germany; [6]DOT GmbH, Rostock, Germany
Corresponding author. Email: schlosser@uni-greifswald.de

Biomaterial implantation causes an acute and chronic inflammatory response at the implant site mediated by different immune cells like macrophages and lymphocytes. These reactions, characterized by broad individual variability, can influence biocompatibility as they affect implant integration and biofunctionalization. For comparative analysis of local inflammation as part of in vivo biocompatibility testing, a model is presented combining simultaneous implantation of different samples into the same experimental animals with morphometry for quantitative results. Up to 4 different implants were simultaneously implanted intramuscularly in rats. Periimplant tissue samples were retrieved after 3 time points from randomly selected animals. Immunohistochemically stained inflammatory cells were counted by digital image analysis. This approach was evaluated and refined using Ti plates with A) 2 different calcium phosphate (CaP) layers, B) 3 different plasma-polymerized allylamine (PAAAm) films, and C) 2 different phospholipid coatings. Examination of CaP layers revealed that numbers of total and tissue macrophages, total T cells and antigen-presenting cells on days 7, 14, and 28 decreased or remained constant for hydroxyapatite but increased for brushite. Evaluation of PPAAm samples, with day 56 instead of day 28 as last experimental day, showed that different plasma parameters caused varying film properties resulting in differences in the tissue reactions. Using the same study period, phospholipid samples were comparable to controls for all examined cells. Thus, activated NK cells, activated T cells, mast cells and fibroblasts were subsequently analyzed and revealed higher reactions for phospholipid samples regarding NK cells, mast cells, and fibroblasts. The results demonstrate that simultaneous implantation is a suitable design for comparative examination of local inflammatory reactions as it reduces individual variability and animal number. Furthermore, digital image analysis was found to be invaluable for quantitative analysis and should be standard for histology in biomaterials research. The chosen study period (7, 14, and 56 d) and panel of cell types (macrophages, T lymphocytes, antigen-presenting cells, NK cells, mast cells, and fibroblasts) provide a comprehensive and customizable format for analysis of short- and long-term local inflammation after implantation. This model should be useful for a wide range of materials.

Poster Session

Nanotoxicity and Embryonic Development

M Tussellino[1], N De Marco[1], S Fusco[2], P Netti[2], R Talevi[1], R Carotenuto[1,*]
[1]Department of Structural and Functional Biology, University Federico II and [2]Center for Advanced Biomaterials for Health Care, Italian Institute of Technology, Naples, Italy
Corresponding author. Email: rcaroten@unina.it

The use of nanoparticles (NP) for different applications such as cosmetics, clothing, food, and drug delivery has been increasing. In particular, the NP used in medicine have a unique opportunity to overcome the cellular barrier to direct molecules to specific targets, for example, drugs used in chemotherapy. To promote the proper development of these nanotechnologies, it is essential to clarify the potential consequences to human health associated with exposure of the NP. The main concern is the small size of nanoparticles and the possibility that they can be internalized in a manner not appropriate in relation to their physical and chemical properties and the nature of the target cells. The use of a *Xenopus laevis* model system, allows us to test the possible toxicity of nanoparticles in vivo, and to evaluate the effects on embryonic development. For this reason we used 2 different protocols of administration, microinjection in the early stages of development and the contact phases. New type of polystyrene nanoparticles (PSNP) of 48 and 100 nm, were microinjected into one blastomere of 2-cell stage embryos. The morphology, the mortality rate and the expression of the mesoderm markers were analyzed. Embryos from the stage 35 to 36 were exposed to different concentration of 20-nm NP already used in medicine (Au, Ag, Fe_3O_4, and SiO_2). All embryos treated present malformations of the head, gut, and tail. Moreover embryos treated with NP of Au, Ag, Fe_3O_4, and SiO_2 showed specific malformations of the skull cartilage and the respiratory system. All treated embryos have a shorter body length compared with the wild type control, particularly those treated with SiO_2. Only the embryos treated with Ag NP or microinjected with PSNP have a high mortality rate. These preliminary data suggest a toxic but not lethal effect on the embryos treated under these conditions.

Delivery of Growth Factors by Gelatin Microspheres in Experimental Heart Failure

N Castaldo[1], MG Monti[1], G Imparato[2,3], A Luciani[2,3], L Guarino[1], L Lippo[1], PA Netti[2,3], A Cittadini[1], L Saccà[1]

[1]Department of Internal Medicine and Cardiovascular Sciences and [2]Interdisciplinary Research Centre on Biomaterials (CRIB), University Federico II and [3]Center for Advanced Biomaterial for Health Care @CRIB, Naples, Italy
Corresponding author. Email: mariagaia.monti@unina.it

Strategies to prevent adverse left ventricular remodeling after myocardial infarction have included several traditional approaches and novel cell-based or gene therapies. Delivery of growth factors in postinfarction heart failure has emerged as a valuable alternative strategy. Our aim was to investigate the effects of sequential release of Vascular Endothelial Growth Factor (VEGF) and insulin-like growth factor-I (IGF1) from biodegradable gelatin microspheres in experimental heart failure. Rats with moderate myocardial infarction (MI) were randomized to receive empty microspheres, microspheres loaded with IGF1 or VEGF, or a combination thereof. Myocardial injections of microspheres were performed at the time of surgery and treatment lasted 4 wk. Echocardiography, LV catheterization, morphometric histology and immunohistochemistry, and molecular assessment of downstream mediators (Akt, eNOS, SERCA2, and others) were performed at the end of the treatment period. Infarct sizes were 33% ± 2%, 28% ± 4%, 24% ± 3%, and 16% ± 3 % in the MI, IGF1, VEGF, and combination group, respectively. IGF1 attenuated LV remodeling, improved LV systolic and diastolic

function, increased myocyte size and reduced apoptotic deaths, capillary loss, and indexes of inflammation. VEGF-treated animals displayed a marked myocardial neoangiogenesis that led to the formation of mature vessels if combined with IGF1 delivery. Downstream effects of IGF1 were principally mediated by the Akt-mTOR dependent pathway, and both growth factors, particularly VEGF, induced a robust and sustained increase of eNOS. IGF1 and VEGF exerted complementary therapeutic effects in postinfarction heart failure. Biodegradable gelatin microspheres provide sustained and controlled growth factor release locally exposing myocardial tissue without the side effects of systemic administration. Our results confirm and expand on the concept that combining biomaterials and appropriate delivery of growth factors represents a simple and effective method to treat ischemic heart disease. Further studies are needed to verify the validity of biodegradable microspheres dual growth factor delivery in larger animal models, before implementing clinical trials.

Safety Assessment of a Gadolinium Liposomal Formulation

A Coppo, C Canapè, R Celeste, S Bussi, A Maiocchi, M Ferraretto

Bracco Imaging Spa, Bracco Research Center, Colleretto Giacosa, Italy
Corresponding author. Email: alessandra.coppo@bracco.com

Gd-loaded liposomes represent an innovative diagnostic tool, yielding a marked contrast in the MR images of the regions in which they distribute. Gadolinium-based contrast agents currently used are administered as concentrated solutions. As yet, there is no formulation based on liposomes loaded with a Gd agent that may find applications in angiographic studies or as reporter of changes in vascular permeability in solid tumors. Gadolinium liposomal formulation (GLF) is a system based on a liposome containing a concentrated solution of Gd-HPDO3A. Our goal was to assess the toxicological profile and safety pharmacology of GLF using the maximum tolerated dose (MTD) and the biodistribution after single intravenous administration to rats and evaluating behavioral, neurologic, and autonomical responses, in either absence or presence of provoking stimuli (Irwin method) after single intravenous administration to mice. MTD study was performed administering GLF at doses of 0.4 and 0.6 mmol(Gd)/kg. Clinical signs, body weight, hematology, and histopathology (lungs, skin, spleen, kidneys, and liver) were evaluated. No animals died during the study. No clinical signs were observed. A decrease in body weight gain was observed in group 2 males (0.6 mmol(Gd)/kg) in comparison to group 1 males (0.4 mmol(Gd)/kg). No changes in hematological parameters were noted. Lipidic vacuoles in liver and spleen macrophages were the most remarkable histopathologic finding. These vacuolated cells were not completely cleaned up after the 2-wk treatment free period. In the biodistribution study GLF was administered at a dose of 0.2 mmol(Gd)/kg and rats were euthanized at different time points, up to 7 d after administration. Blood, liver, spleen, skin, and femur were collected for ICP-MS determination of Gd content (reported as percent of injected dose). In blood, the maximum value was observed 30 min after dosing (100%). Maximum values in liver (21%), spleen (12%), and skin (0.3%) were observed 48 h after dosing and decreased 7 d after treatment (30 to 47% less). These findings suggest that Gd is slowly eliminated, especially in liver and spleen. In the Irwin study mice were administered with GLF (0.2 mmol(Gd)/kg). A control group was administered with physiologic solution. No significant differences were noted between mice treated with GLF and controls. Based on these results, GLF is not expected to cause alterations in behavioral, neurologic, and autonomical responses.

Comparative Staining Technique for Calcified Bone with Metallic Implants

TS Goia*, KB Violin, JC Bressiani, AH Bressiani

Material Science and Technology Center, IPEN, São Paulo, Brazil
Corresponding author. Email: tamigoia@gmail.com

Unveiling the growth pattern of bone tissue after implantation, may help the development of implants with specific features, besides establishing recovery time for each step during bone repair. We aimed to identify the bone remodeling in rats during repair after surgical implantation of porous metallic implants with histologic approach over calcified bone-implant samples using different staining methods. Implants of Ti-13Nb-13Zr alloy with mean porosity of 30% and cylinder shaped were surgically positioned in the femurs of 10-wk-old male Wistar rats. The animals were euthanized on day 28 and bone-implant samples were fixed with formalin solution, the blocks of calcified tissue samples were prepared using methyl methacrylate resin and the histologic slides from the blocks were performed using a cutter and finally trimmed with sandpaper to achieve the desirable thickness to perform staining and further analysis. The histologic evaluation were made using bright field of unstained (U) slides and hematoxilin-eosin (HE), toluidine blue (TB), and Masson trichrome (MT) stained slides. To perform all the staining and analysis of the slides the resin was not washed out as with paraffin preparations. The morphologic analysis was performed by optical microscopy, which followed from U, MT, HE, TB. Each of these preparations showed different features of bone repair. From all techniques the osteocyte nucleus was always distinct and recognizable even with U slides, due to the resin impregnation and thickness of the slides. In comparison to the 3 staining methods used the HE marked well the differences between the osteocytes nuclei in purple and the surrounding bone matrix in pink. For the MT stained slides the bone matrix got stained by 2 shades of blue, light blue for a new formed bone, and dark blue for old bone, which pointed out the grade of bone remodeling that took place after implantation. From TB slides the highlight was the metachromasia in bone matrix, which presented as pinkish color in contrast to the blue staining of other structures. Besides differentiating the several stages of bone maturation and remodeling, it was possible to evaluate the cell migration and differentiation towards the center of the cylinder-shaped metallic implant as well as the intimacy of the bone-implant interface. The content of the bone-implant interface without fibrous capsule that could be seen with the use of HE, MT, and TB showed a successful osteointegration.

Electrospun Grafts as Guide for Peripheral Nerve Regeneration: Design and In Vitro Validation

V Guarino*, MA Alvarez-Perez, V Cirillo, L Ambrosio

Institute of Composite and Biomedical Materials, National Research Council of Italy, Naples, Italy
*Corresponding author. Email: vguarino@unina.it

Continuous biomaterial advances and the regenerating potential of the adult human peripheral nervous system offer great promise for restoring full function to innervated tissue following traumatic injury via synthetic nerve guidance conduits (NGC). An efficient promotion of nerve regeneration is assured by the use of engineered scaffolds with morphologic and chemical properties which mimic the natural microenvironment of the healthy nerve. Recent studies just have successfully proven that fibers are capable of directing both in vitro and in vivo peripheral neurite outgrowth. Fiber meshes with diameters at the size of a cell or smaller (< 20 μm), produce faster neurite outgrowth due to their similarity to the natural ECM fibrous organization. Likewise, the combination of polymers exhibiting specific bioactive moieties, can promote favorable interactions with neuronal cells in terms of adhesion and nerve outgrowth. Here, we propose the design of tubular platforms by electrospinning technique to realize modular structures as guide for nerve regeneration. Bilayer tubes were obtained by the overlapping of fibrous shells with peculiar morphologic (that is, size, anisotropy) and biochemical cues (that is, gelatin inclusion; Figure 1): the luminal layer made of PCL and Gelatin is composed of longitudinally aligned nanofibers collected onto a mandrel with controlled rotating rate (1000 to 3000 rpm). The outer layer, randomly organized nanofibers made of PCL, was obtained by wrapping fibers onto the previous layer until forming the bilayer system. The interaction with human mesenchymal stem cells (hMSC) was investigated in terms of cell viability and neurogenic differentiation. Late cellular events, such as neurite outgrowth, were ultimately studied by specific biologic assays (RT-PCR and immunocytochemistry). PCL micro and PCL/gelatin nanofibers have been assembled to form tubular grafts with tunable morphology by the fine setting of process parameters (that is, polymer/solvent coupling, polymer concentration, flow rate, and electric field) and tailor-made electrospinning setups. We demonstrated that cells feel the morphologic signals due to either fiber size scale or anisotropy, as confirmed by differences in early cell activities (that is, viability). Moreover, the presence of biochemical cues strictly embedded into the fibers contribute to amplify the guidance effect of nanopatterning thus assuring the advance of late cell activities (that is, cell differentiation, neurite outgrowth, and others) over 14 d of culture.

New Approaches to Tissue Regeneration in Chelonians

E Merli[1,*], F Di Ianni[2], I Pellizzone[3], F Burtini, V Conti[1], E Scaltriti[1], R Ramoni[1], GP Squassino[3], M Del Bue[2], S Grolli[1]

[1]Department of Animal Production, Veterinary Biotechnology, Food Quality and Safety and [2]Department of Animal Health, School of Veterinary Medicine, University of Parma; and [3]Veterinary Practitioner, Parma, Italy
*Corresponding author. Email: elisa.merli@tin.it

Turtles, both in nature and in captivity, are often subject to traumatic injuries as a consequence of predators' attacks, bites from other animals, bad fallings, car crashing, and lawn-mowers' cuts. Whatever the cause that has wounded limbs, head, or carapace, healing is often impaired by the slow tissue recovery typical of these animals. Current therapies are based on the use of nonorganic materials, such as resins, screws, nails and/or wires for the treatment of the carapace, while bandages or dressing are applied to soft tissues wounds. Unfortunately, these materials can sometimes create issues in terms of tolerability and delay in tissue healing. The aim of this research was the application of nonconventional techniques to soft as well as hard tissue injuries of chelonians, in order to support and/or replace conventional therapies. In particular, our efforts have been addressed to the optimization of a protocol for the preparation of thrombocyte concentrates (TC), the equivalent of mammals Platelet Rich Plasma (PRP), starting from chelonians blood samples. Platelet rich plasma is a platelet derivative rich of growth factors involved in the processes of tissue repair, where they play a key role in cell migration, proliferation and differentiation. Furthermore PRP has an antimicrobial effect and reduces pain, thus improving animal welfare. Platelet concentrates are routinely used in mammals for the treatment of both soft and hard tissue lesions. As a further objective of our research, we have set up protocols for TC application in chelonians as solution, gel, or spray, depending on the therapeutic indications. To our knowledge the literature does not report protocols for preparation of TC in reptiles. The preparation of TC necessarily differs from protocols developed for PRP preparation in mammals, since reptile thrombocytes and erythrocytes are both nucleated. Thrombocytes partitioning into the plasma fraction has required a 2-step centrifugation protocol, leading to the recovery of 41.4% (n = 11, SE = 3.5) of whole blood thrombocytes. The TC preparation has been successfully applied on different clinical cases. In particular it has been applied both as gel and spray in head and limb lesions and in a few cases of carapace damage, with consistent tissue loss and exposure of internal organs. Although a larger number of clinical cases are needed before conclusive results can be drawn, the outcomes so far obtained are strongly encouraging.

Direct Nanoparticle-Protein Interactions Revealed Using the hERG Potassium Channel as a Target for the Screening of the Environmental and Cytotoxic Effects of Engineered Nanoparticles

S Piscopo, ER Brown

Department of Animal Physiology and Evolution, Stazione Zoologica Anton Dohrn, Naples, Italy
*Corresponding author. Email: euanbrown2@gmail.com

Despite increasing use, little is known of the impact of engineered nanoparticles on aqueous systems. This knowledge-gap represents a major challenge as the cytotoxicity of these molecules is largely unknown and they are undetectable in environmental samples by standard laboratory methods. Here, we report results at the cell membrane level using a highly defined medium throughput system used in hit-lead pharmacology. Human embryonic kidney (HEK) cells were transfected with the human ether-à-go-go related gene (hERG) which codes for the Kv1.1 potassium ion channel. This intramembrane protein contributes to the repolarizing phase of the cardiac action potential in active heart muscle. Transfected cells were subjected to whole-cell voltage clamp at –70 mV. An automated protocol tested membrane integrity, capacitance, and the outward and tail currents of the hERG channel. Cells were superfused at 2 mL/min with a sys-

tem that permitted vibration-free changes of superfusate solution without sedimentation of NP. We screened engineered dialyzed filtered SiO_2 nanoparticles of 30 nm and dialyzed ZnO of 30 nm at concentrations from 1 to 100 µg/mL. In controls, membrane integrity, cell capacitance, leak, and hERG currents remained stable for up to 1 h under whole cell voltage clamp. In 10 µg/mL SiO_2 membrane capacitance and current was unchanged while ZnO NP at the same concentration caused a significant and reversible decrease in activation of the hERG current that was consistent with a positive charge interaction with the inactivation gate. This effect was removed by preincubation of NP in 1% BSA. We conclude that ZnO NP are likely to have a direct action on the channel gating either by slowing inactivation or by increasing the rate of opening of the channel. At higher concentrations, both NP cause membrane breakdown (presumably by pore formation). This specific action at low concentrations underlies the importance of well-defined assay systems in assessing toxicology of NP and indicates a protective role for serum proteins.

Invertebrate Models to Test In Vivo Advanced Materials for Biomedical Application

V Marchesano[1,*], A Ambrosone[1], T Pellegrino[2], C Tortiglione[1]

[1]Istituto di Cibernetica E Caianiello CNR, Pozzuoli, Italy; [2]Italian Institute of Technology, Genova, Italy
[*]*Corresponding author. Email: v.marchesano@cib.na.cnr.it*

Due to their superior brightness, higher photostability, and narrower spectral emission compared with conventional organic fluorophores, spherical and rod shaped fluorescent semiconductor nanocrystals, also known as quantum dots (QD) or quantum rods (QR), are increasingly being used to probe biomolecular interaction in living cells, to study intracellular processes at single-molecule level, high resolution cellular imaging, as well as for long-term in vivo observation of cell trafficking, tumor targeting, and diagnostic. Evidences are cumulating that nanoparticles plays active roles even in absence of specific ligands and that factors such as size and charge are crucial for activation of cell responses internalization and intracellular trafficking. Live studies in higher vertebrates relying on the injection of nanoparticles into the bloodstream are limited by the opsonization process, namely the coating of nanoparticle surface by components of the circulation, such as plasma proteins. This process renders the particle recognizable by the the reticulo-endothelial system (RES), which provides to their phagocytosis. Despite all efforts, however, complete evasion of the RES by these coated nanoparticles has not yet been possible and alternative in vivo systems to study the cellular response to unfunctionalized nanoparticles are needed. At the base of metazoan evolution, Cnidaria have been shown amenable systems to study the nanoparticles/living system interactions. Hence, by using 2 model organisms, the polyp *Hydra vulgaris* and the sea anemone *Nematostella vectensis*, we assess in vivo the relationship between amino-PEG coated CdSe/CdS core/shell QR and cell uptake. By manipulating the surface charge at different pH, we tuned the capability of *Hydra* ectodermal cells to uptake QR. Due to extreme photostability of the nanoparticles, tracking QR labelled ectodermal cells over long periods of times led to the discovery of new migration dynamics and intercellular trafficking events in the tentacles and subhypostomal region, monitored and characterized both in normal growth and regeneration conditions. Moreover, here we monitored the efficient labeling of *N. vectensis* along all developmental stages, from unfertilized egg, to the planula, primary polyps, and adult life, showing the feasibility of using innovative biologic probe to investigate in an unprecedented way biologic phenomena.