Biotechnology

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Introduction

The guidelines of the Biotechnology Program are research and development aiming at developing and manufacturing products of pharmaceutical interest. This Program has two main research areas, namely Pituitary Hormones and Biopharmaceuticals

The first one comprises a group with a long experience on Recombinant Human Pituitary Hormone synthesis, purification and characterization. Up to now, they have worked mostly with human growth hormone (hGH), human prolactin (hPRL), human thyrotropin (hTSH), human follicle stimulating hormone (hFSH) and human luteotropin (hLH), with a particular emphasis on glycoprotein carbohydrate structures. An important research line is devoted to Growth Hormone Gene Therapy, working mostly on animal models: immunocompetent and immunodeficient-dwarf mice. For several years, this development has been based on ex vivo grafting of transduced keratinocytes, while more recent results, that are very promising, have been obtained with the injections and electroporation of naked plasmid DNA. Besides research, they also have activities in the Biotechnological Production and Downstream Processing of the same recombinant hormones, which are produced in both E. coli and mammalian cells and in the development of joint-ventures with the National Industry. The biological effects of radiation are studied using non-tumoral and tumoral (mammary, prostate, melanoma, thyroid) in vitro models considering also the application of hTSH to thyroid cancer diagnosis and treatment with ^{131}I. The Center is also currently involved on genotoxicity assessment of radiopharmaceuticals produced by IPEN.

The Biopharmaceutical area is dedicated to the research of isolation, structural analysis and biological activities in different biological systems of macromolecules. These macromolecules are peptides or proteins, either native or recombinant with medical or pharmaceutical interest. During this period, new proteins related to serine protease activity, breast cancer development and angiogenesis, were described. The effects of ionizing radiation on macromolecules have also been investigated to detoxify animal venoms in order to improve antigens for antisera production, or even modify microorganisms for vaccination. Recently, we started investigating the peptide fractions of several venoms, identifying many serine-protease and metalloprotease inhibitors. Ideally, these inhibitors will be co-crystallized with the target enzyme, aiming to characterize the inhibitor-enzyme interaction. Such data could provide knowledge to develop new drugs against coagulopathies and other endogenous protease related diseases.

The Animal Laboratory Division of IPEN is responsible for the breeding and production of small laboratory animals. In this facility Specific Pathogen Free (SPF) animals are bred and maintained, under controlled sanitary conditions, to be used for testing of the radioisotopes production and research. This facility also produces different mutant mice, severely immunodeficient mice and their offspring, besides other mice lineages as well as normal rats.

Pituitary Hormones

The research and production activities related to the five human pituitary hormones, namely growth hormone (hGH), prolactin (hPRL), thyrotropin (hTSH), follitropin (hFSH) and luteotropin (hLH), still constitute the basic working field of the group. Our goal is the development of applied research, especially emphasizing the interaction between the Academic and the Industrial worlds, an aspect that has been neglected for so long in Brazil. Under this aspect, a partnership with the company Biosynthesis has been started in that period generating an important project PIPE-FAPESP. We continued our main collaborations with the Aarhus University (Denmark) and with clinicians from FMUSP, especially in the field of Gene Therapy, with the groups of Genetic Ichthyology and the Faculty of Pharmaceutical Sciences of USP for the purpose of cloning Arapaima gigas (Pirarucu) gonadotropins, and with the Department of Physiology and Biophysics of the Institute of Biomedical Sciences (ICB/USP) for studies related to prolactin and growth hormone. As always, the main emphasis of the group has been given to scientific production and to collaboration with well-known national and international research groups. Thus, in this 3-year period, 16 scientific papers have been published all in international journal whose impact factor was always between 1 and 6.4, and 2 Meeting Abstracts published in journals of high impact.

Human growth hormone (hGH)

Human growth hormone (hGH) production and quality control has been already established at the laboratory level and, as stated several times, is only waiting for the "industrial decision", that unfortunately is frequently independent from the researches. However, an important research line has being carried out developing alternative ex vivo and in vivo Gene Therapy strategies for phenotypic correction of dwarfism, using the human (hGH) and the mouse (mGH) genes. In these studies, two animal models available at our animal facility, the immunocompetent dwarf (lit/lit) and the immunodeficient dwarf (lit/scid) mice, are employed. The goal of this approach is the development of an animal model, based on these dwarf mice, in which it can be possible to obtain useful and sustained circulatory levels of growth hormone with phenotypic corrections, such as body weight gain and longitudinal growth. Our researches are thus moving closer to pre-clinical testing. More recently, the efforts were concentrated on the use of an in vivo system based on naked hGH DNA administration followed by electroporation in the quadriceps muscle of lit/scid mice. In a previous work, we related a sustained secretion of hGH during a 60-day assay together with a highly significant increase in the body weight of these animals. In this period, we also developed a novel homologous model, in which the mGH gene was electrotransfered to the immunocompetent mice (lit/lit), a condition more similar to that of GH-deficient children (Cecchi CR et al., 2014). The results of this work also confirmed the feasibility of the proposed treatment, both in terms of higher circulatory levels of the main effector of GH, the insulin-like growth factor I (IGF-I), and the absence of anti-GH antibody formation. In a recent work (Higuti E et al., 2016), we set up a more effective pre-clinical assay in pubertal dwarf (lit/scid) mice treating 40-day old animals injected with hGH-DNA into the non-exposed tibialis cranialis muscle, via a less invasive approach. This treatment provided, for the first time, IGF-I concentrations at the same level as co-

aged scid mice 15 days after administration in pubertal mice, as well as catch-up growth increases of the order of 77% for the femur length. An example of dissected femurs of hGH-DNA-treated mice is shown in Fig.1. A more detailed study comparing DNA administration into the quadriceps or tibialis muscle of lit/scid mice under different injection conditions (exposed or non-exposed muscle) and electrotransfer parameters was also carried out. Our data showed that hGH-DNA administration into non-exposed tibialis muscle was an equally efficient, less traumatic treatment, much more suitable for the pre-clinical testing than injection into exposed quadriceps. During this work, a more precise methodology for femur length determination based on initial and final radiographic measurements of the same animal was set up and these results were accepted for publication in Current Molecular Medicine (Cecchi CR et al., 2017). We are starting a new project in which we intend to use our experience with electrotransfer to minimize the effects of osteogenesis imperfecta. This is a congenital connective tissue dysplasia, known as brittle bone disease, mainly characterized by bone deformity, brittleness and low density, short stature, and other connective tissue changes associated with structural or quantitative modifications of collagen. The effects of GH have already been reported with an animal model with osteogenesis imperfecta, the "oim mice", after administration of recombinant hGH, obtaining a decrease of the fragility and an increase of bone density, with consequent decrease in the number of fractures, increased weight gain, femur and animal lengths. Therefore, our main objective is to study, for the first time, the effects of gene therapy by electrotransfer of the mGH gene in the oim mice, which were already acquired from The Jackson Laboratories and are being maintained in our animal facility. We also can emphasize the continuity of our participation to the most prestigious International Meetings in this area, such as the 18th Annual Meeting of the American Society of Gene and Cell Therapy, in New Orleans, 2015, in which we participated with one abstract published in the Molecular Therapy. These studies are being carried out in collaboration with the Endocrinology Division of the FMUSP/São Paulo and with the Department of Biomedicine at Aarhus University/Denmark. In collaboration with ICB-USP researchers, the distribution of GH responsive cells was mapped and the receptor involved in the central effects of GH was identified. Our findings deepen the understanding of hGH signaling in the brain and suggest that central GH signaling is likely more ample and complex than formerly recognized and resulted in a publication in Brain Structural Function (Furigo IC et al., 2017).

Figure 1. Dissected right femurs of hGH-DNA-treated and saline-treated lit/scid and of untreated scid mice during a 6-month bioassay. ls, untreated lit/scid; sc, non-dwarf scid mice; s, saline-treated lit/scid; p, plasmid DNA-treated lit/scid.

Human pituitary glycoprotein hormones

Human pituitary glycoprotein hormones include thyrotropin (hTSH), follitropin (hFSH) and luteotropin (hLH), all heterodimers formed by an alpha and a beta subunit. hTSH is related to thyroid function and metabolism, and is used in the diagnosis and therapy of thyroid cancer, while hFSH and hLH are mostly used for the treatment of human infertility. These recombinant products are among those with the highest aggregate value, their purified forms reaching prices up to US$ 12.000/mg! Considering their carbohydrate moiety, which is strictly related to their in vivo bioactivity, these proteins must be synthesized in mammalian cells. Being CHO cells the most commonly used for their industrial production. Our laboratory has synthesized and characterized hTSH, having also the know-how for synthesizing hFSH and hLH. The effects of butyrate and manganese on productivity, sialylation, N-glycosylation site occupancy and biological properties of CHO-derived hTSH were evaluated, showing no evidence of alterations in its bioavailability, although increases in hTSH production (up to 3-fold), in sialylation (up to 14%) and site occupancy (up to 3%) occurred. In 2014, these data were presented in the European Biotechnology Congress, in Italy, and published in the Journal of Biotechnology (Damiani R et al., 2014). During this period (2014-2016), the laboratory completed the studies concerning to the substitution of animal-based bioassays by alternative methodologies for hFSH and hTSH potency assessment. A reserved-phase high performance liquid chromatography (RP-HPLC), developed by us, was compared to the in vivo bioassay for hTSH potency determination (Almeida BE et al., 2014). The results demonstrated that this physical-chemical method is a novel and viable alternative for avoiding or reducing animal use. In the same research field, to assess potency determination of human erythropoietin, a sialoglycoprotein that stimulates the erythropoiesis, we investigated an in vitro cell proliferation assay, which was applied in conjunction with a RP-HPLC methodology for the determination of sialic acid content and compared to the results of an in vivo bioassay (Machado FT et al., 2016). The in vitro assay resulted in a non-significant lower mean difference of the estimated potencies. Glycosylation sites of hTSH are also studied since these sites are not always occupied and occupancy is related to folding, trafficking, initiation of inflammation and host defense, as well as congenital disorders of glycosylation (CDG). For the first time, N-glycoprofiling analysis was applied to the site-occupancy determination of two native pituitary hTSH, in comparison with three recombinant preparations of hTSH. The results showed that the occupancy and carbohydrate mass can be up to 34-57% higher in recombinant hormones. We believe that this kind of comparison is extremely important when characterizing a widely used recombinant biopharmaceutical. These results were recently published in the International Journal of Molecular Sciences (Ribela MTCP et al., 2017). During this period, we also started using a strain of human embryonic kidney cells (HEK293) for the synthesis of hTSH, since recombinant biopharmaceuticals produced in these appropriate human cell lines are expected to present glycosylation profiles more similar to their human counterparts and less immunogenicity. The produced hTSH-HEK, compared to a CHO-derived recombinant and to a pituitary-derived preparation, was considered to be suitable for clinical applications, in view of its human origin, biological activity and particular carbohydrate composition (paper submitted to Applied Microbiology and Biotechnology).

A work was also carried out in collaboration

with The Agency for Agribusiness Technology of Piracicaba (SP) testing different commercial preparations of equine chorionic gonadotropin (eCG) concerning ovarian stimulation in cattle (Alvarez RH et al., 2016).

Pirarucu (Araipama gigas) pituitary gonadotropins

Araipama gigas (pirarucu) is a giant fish native to the Amazon River basin that can reach up to 2 meters in length and in weigh up to 150 Kg. This species is in danger of disappearing because of the exploitation by the fishing industry and increasing human presence. It is largely used for food and extractivism and commercial breeding is still incipient. In the last period (2011-2013), our research group has isolated, from the pituitaries, and characterized the cDNA of the α-subunit of A. gigas (ag) gonadotropins (FSH and LH), carrying out also a phylogenetic analysis based on its amino acid sequence. During this period (2014-2016), the cDNAs of ag-FSHβ and ag-LHβ-subunits have been isolated, characterized and phylogenetically analysed. These results, including a three dimensional comparative modeling of ag-FSH and ag-LH, were submitted to publication and are under revision by the Journal Plos One. The biotechnological synthesis of these hormones, useful for physiology and fertility applications, will be carried out with basis on these isolated genes.

Prolactin (PRL)

Prolactin is a 23 kDa protein hormone secreted by the anterior pituitary. Its therapeutic use is still limited, while it is important for diagnostic applications such as prolactinomas, infertility and tumorigenesis. In the context of recombinant hormone production, the group has been developing the expression, purification and characterization of human prolactin and its analogs in a research laboratory scale. In this period, the receptor antagonist G120R-hGH was developed. A publication in "Protein Expression and Purification" (Menezes ACSC et al., 2017) describes the synthesis and characterization of G120R-hGH secreted into bacterial periplasm and obtained with a vector based on a constitutive lambda PL promoter. This antagonist can be useful for studies aiming at investigating the effects of a simultaneous inhibition of GH and prolactin signaling, as a potential anti-tumoral or anti-diabetic compound. Studies related to glycosylated hPRL (G-hPRL) were published in Journal of Biotechnology (Capone MN et al., 2015). The N-glycan structures present in native pituitary G-hPRL were determined and compared with those present in the recombinant hormone. To obtain recombinant G-hPRL, genetically modified Chinese hamster ovary cells (CHO) adapted to growth in suspension were treated with cycloheximide, thus increasing the glycosylation site occupancy from 5.5% to 38.3%, thereby facilitating G-hPRL purification. N-Glycan profiling proved to be a useful and accurate methodology also for monosaccharide determination, molecular mass and carbohydrate content determination for the two G-hPRL preparations, in good agreement with the values obtained directly via MALDI-TOF-MS.

Cellular response to ionizing radiation

Ionizing radiation is a physical agent known to induce mutation and cancer, being also used as a widespread therapeutic modality for cancer treatment. Thus, one of the challenges in radiobiology and oncology is to understand how the cells respond to radio-induced stress because of exposure to radiation and its outcomes. The group, aiming to assess radio-induced cytogenetic damage/mutagenesis has

developed studies focusing some interlinked aspects. The objective of the cytogenetic consists of a comparative study of the effects of different radiation types and radionuclides of medical interest (^{153}Sm, ^{177}Lu, ^{131}I, ^{18}F, ^{68}Ga), in human and rodent cells, by cytogenetic and biochemical techniques. The second aspect of our study consists in the establishment of dose-response curves for different types of radiation (γ, β and neutron) for biological dosimetry (dosimetric aspect) directed to the quantitative estimate of absorbed dose, according to the criteria adopted by IAEA (2001). The calibration curves for the γ radiation of ^{60}Co and ^{137}Cs, for the β radiation of ^{90}Sr and for fission neutrons produced in the Reactor R1 of IPEN-CNEN/SP have already been established by assessment of chromosome aberrations, micronucleus and comet assays An improved in vitro micronucleus assay for biological dosimetry was introduced in our laboratory utilizing fluorescent staining technique on human tumor cells (MCF-7) treated with a nitric oxide inhibitor and irradiated (^{60}Co). This showed that nitric oxide inhibition could be a radio sensitizing approach in tumor therapy, increasing radio induced genotoxic damage and reducing cell viability and clonogenic potential. A third aspect (therapeutic approach) consists in evaluating the cytogenetic effects of radiopharmaceuticals used in nuclear medicine, e.g. ^{131}I, administered to patients with differentiated thyroid carcinoma (DTC), with or without the use of recombinant human thyrotropin (ThyrogenR or rhTSH) (project approved by FAPESP), was carried out in collaboration with the Nuclear Medicine Center of FMUSP. A study also using human thyroid cancer cells (WRO) was published for comparing the radiosensitivity between target cells of radioiodine (thyroid cells) and peripheral lymphocytes of patients with DTC. Included in this research area is also a collaboration study joining researchers from the Centers of Biotechnology and Radio pharmacy and the Biosintesis Laboratory for pre-clinical trials of radiopharmaceuticals produced at the Radiopharmacy Center (IPEN-CNEN/SP). This work was supported by an institutional grant, and the final results were published. After these achievements, our Laboratory is currently working on a flow-cytometry based methodology to micronucleus scoring, with some relevant results (Fig. 2). This improvement will reduce dramatically the duration of genotoxicity studies.

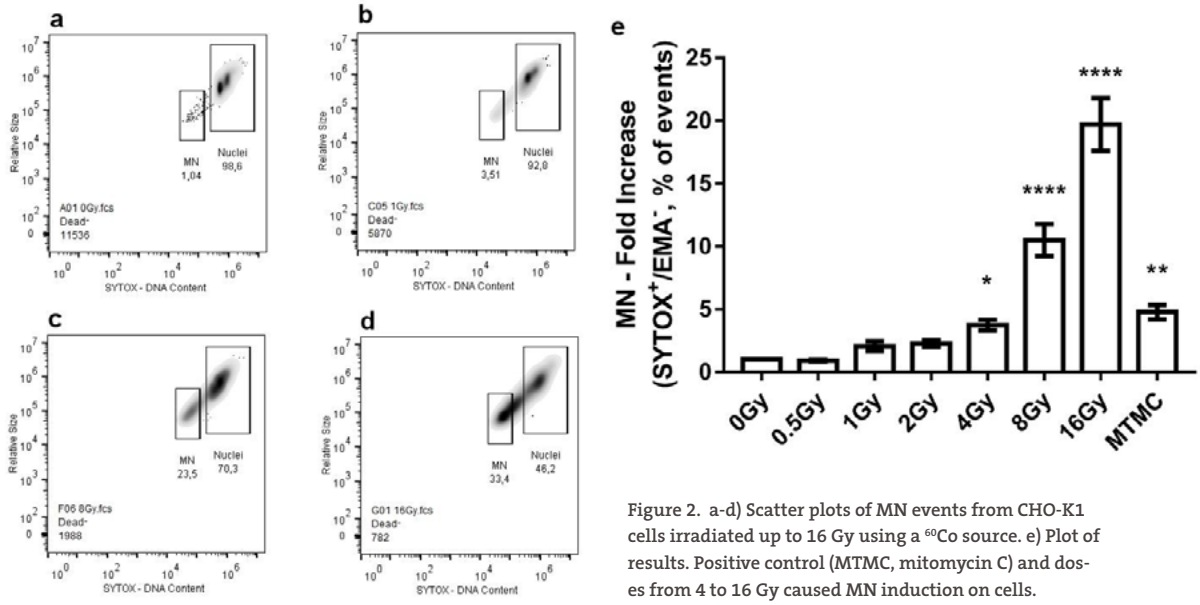

Figure 2. a-d) Scatter plots of MN events from CHO-K1 cells irradiated up to 16 Gy using a ^{60}Co source. e) Plot of results. Positive control (MTMC, mitomycin C) and doses from 4 to 16 Gy caused MN induction on cells.

Our research also includes the study of radio modifiers from natural sources, such as resveratrol (in collaboration with Chemical and Environmental Center) and propolis. The study about the radio modification effect of propolis and their HPLC purified fractions (in collaboration with a Japanese group) in normal and human cancer cells is in progress, having already published data on the effect of propolis on CHO-K1 cells irradiated with ^{60}Co through the differential staining technique and digital analysis. Using this technique, we could be able to analyze 10-100x more cells than by the usual manual counting. The data obtained via genotoxic and cytotoxic tests and survival curves showed a radioprotector effect of propolis on the induction of DNA damage and cell death and were published. These data indicate a potential promising use of propolis as a natural, non-toxic, effective substance for protection against genotoxic and cytotoxic damages induced by the ionizing radiation.

The most recent research subject of our lab has been the construction of three-dimensional in vitro tumor models, using ferromagnetic iron oxide nanoparticles (Fig. 3). The model will help IPEN and other institutions to study cancer physiology on models closer to animal models.

Protein Radioiodination

The Group of Hormones of the Biotechnology Center has a long and well-known expertise on Protein Radioiodination, mostly with the use of ^{125}I. At least ten studies on pituitary hormone radioiodination were published, before the year 2000, in International Journals of high impact. In the period 2001-2010, nine more papers were published, providing precious collaborations to other Brazilian research groups, in prestigious journals as well. In 2013, a paper was published in Journal of Experimental and Integrative Medicine (Lemos et al.), in collaboration with the Department of Pharmacology of Unicamp (Campinas-SP). One more paper was published now in Pharmacological Research, in collaboration with the Department of Pharmacology of ICB/USP (Rodrigues L et al., 2017). This excellent specialty, in a specific area of the Nuclear Field so long neglected, deserves a proper emphasis, especially considering the work of all the technicians and researchers that dedicated themselves to the study and manipulation of this extremely useful radioisotope.

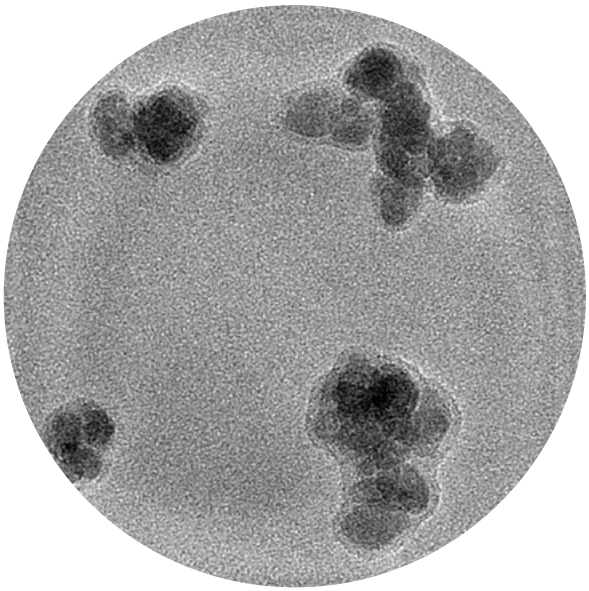

Figure 3. CHO-K1 (top) and LNCap (middle) spheroids cultured using magnetic levitation. Iron oxide particles (bottom) produced in house by our team

Biopharmaceuticals

Identification, isolation and characterization of new compounds of clinical interest, from plants and animal molecules have been developed. Additionally, studies involving ionizing radiation have been done in order to detoxify or even modify molecules mainly to improve antiserum production and vaccines. Many of pre-clinical assays, the main tool employed to characterize the candidate to a new medicine, have now been standardized to get an accreditation.

Cancer cellular and molecular biology

The renal cell carcinoma (RCC) accounts for approximately 2-3% of human malignancies and, among urologic tumors, it is the most aggressive. Clear cell RCC (ccRCC) is the most frequent RCC histological subtype, which is characterized by frequent inactivation of the VHL gene. This mutation confers chemoresistance and radioresistance, making ccRCC one of the most difficult metastatic cancers to treat. Our group has been investigating molecular therapeutic targets, biomarkers and also, mechanisms of radioresistance of ccRCC.

Biological screening of snake venoms and toxins

Snake venoms are an extremely rich source of biologically active substances modulating several aspects of the regulation of homeostasis. The investigation of potential new drugs in biological samples has been a major field of research in many laboratories. This activity has focused part of its efforts in screening and isolating toxins with potential therapeutic uses. Amongst those, toxins from the Brazilian rattlesnake which seem to be involved in the down regulation of the sodium/potassium pump have been investigated. Such molecules have a potential for further understanding the fine physiology of many cell types and for guiding the rationale design of cellular metabolism modulators. Also, toxins with modulatory activity on the blood clotting system (Snake Venom Serine Protease - SVSP) are under investigation. Such toxins might be employed for the treatment of coagulation disorders and as auxiliary drugs in major surgeries where unexpected activation of the blood clotting cascade could put the patient at risk. These proteases are also key players in a wide range of biological processes; for example, in regulating the cell cycle, cell growth and differentiation, affect the haemostatic system, antigen processing and angiogenesis. In addition, it is becoming apparent that the aberrant functioning of certain proteases may be involved in several disease states, including Alzheimer's disease, in cancer metastasis and in inflammation (over-reactive inflammatory reactions in CNS often cause irreversible neuronal damage).Other fields of investigation include the isolation and characterization of snake venom peptides with antimicrobial activity, structurally related to beta-defensins. In the field of antivenoms, a novel anti-coral snake venom formulation, based on Australian venoms produced very promising results, with the new formulation being much more effective than the currently available antivenom produced in Brazil.

Biological evaluation of new products for health

This activity is mainly based on the biological evaluation of substances and biomaterials performing in vitro and in vivo tests. These tests are carried out in compliance with the rules of ISO-10.993 and some other international directives. Such tests include: cytotoxicity, genotoxicity, hemocompatibility etc. and some others tests of systemic toxicity and implants. Synthesis of polymeric biomaterials was also done, resulting in three patents submitted to INPI in 2007. A biomaterial can be defined as a substance (with the exception of drugs) or a combination of substances (either synthetic or natural), employed in the treatment, improvement or substitution of organism tissues, organs or function. Since interaction with the biological system is involved, biocompatibility implies the capability of the material to exhibit in the host the appropriate functional and "biomi-

metic" qualifications. In recent years, interest in biomedical applications of natural and synthetic polymers has grown steadily, with a substantial contribution to the quality and duration of human life. Presently, novel porous biologically active composites based on hydroxyapatite (HA) and poly(caprolactone) (PCL) have been developed and tested, with potential for use in scaffolds for bone tissue engineering. The experiments are focused on the synthesis and biological response of bone to the PCL/HA composite. Such work resulted in a partnership with the Biosintesis Company, which received a financial support from FAPESP (PIPE project).

Recombinant proteins – Refolding from inclusion bodies using high hydrostatic pressure

Until the 1980s decade, the production of proteins for therapeutic and research purposes was obtained by purification from their native sources. The production of proteins was greatly facilitated by transgenic protein expression, overcoming the difficulties of purification of proteins that were present at their native sources usually very contaminated and at low levels. The bacteria Escherichia coli is the most efficient and cost-effective host for recombinant heterologous protein production. However, E. coli is often unable to fully process the recombinant foreign proteins during overexpression and therefore misfolded proteins forms insoluble aggregated proteins in bacterial cytoplasm, known as inclusion bodies (IB). Solubilization of the IB and the posterior refolding of the proteins is necessary to produce active proteins from IB. Utilization of high hydrostatic pressure is a novel and robust method to disaggregate proteins from IB, by solubilization of the aggregates in mild conditions, maintaining the existing native-like secondary and tertiary structure of the insoluble and mostly inactive proteins produced in bacteria. We demonstrated that high pressure can convert insoluble aggregated proteins from IB to preparations with native tertiary structure and fully biological activity with very high yields (Fig. 4). Among the proteins that have been successfully refolded by our group are the non-structural protein 1 (NS1) and envelope (protein E) proteins from dengue and zika viruses, endostatin, green fluorescent protein, a promising protein for Schistosoma mansoni vaccination (Sm29), the pentamer of subunit B of cholera toxin (CTB), among many others.

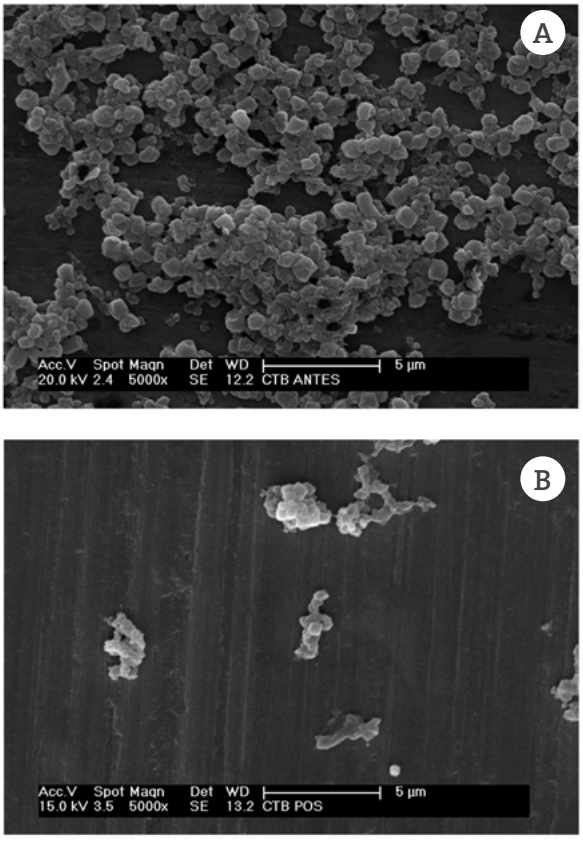

Figure 4. Scanning electron microscopy of cholera toxin expressed in Escherichia coli inclusion bodies before (A) and after (B) refolding with high pressure. Scale 5 µm.

Structural analyses of soluble human protein

The L10 ribosomal protein (RPL10) plays a role in the binding of the 60 S and 40 S ribosomal subunits and in mRNA translation. The evidence indicates that RPL10 also has multiple extra-ribosomal functions, including tumor suppression and its overexpression osteoblasts exhibit a cell autonomous alteration that lead to increased mineralization in vitro. We successfully cloned and expressed full-length human RPL10 (hRPL10) protein and isolated inclusion bodies that had been formed under mild growth conditions. After the hRPL10 purification using a two-step process of non-denaturing protein extraction from pelleted inclusion bodies, we studied the characteristics of this protein using circular dichroism spectroscopy and by monitoring the changes induced by the presence or absence of zinc ions using fluorescence spectrometry. The results suggested that the strategy used to obtain hRPL10 is simple and could be applied to obtaining other proteins that are susceptible to degradation ("A simple strategy for the purification of native recombinant full-length human RPL10 protein from inclusion bodies"). Angiotensin-converting enzyme catalyzes the conversion of angiotensin I to the vasoconstrictor angiotensin II and the hydrolysis of bradykinin (BK). Human somatic angiotensin-converting enzyme has two homologous domains (N and C) that share 60% identity, and the catalytic site of the C-domain exhibits three-fold greater activity than the N-domain in the hydrolysis of angiotensin I in vivo. The catalytic site of C-domain of angiotensin-converting enzyme peptide was expressed in a bacterial system, and its purification was performed in one step. Structural analysis by circular dichroism and fluorescence confirmed that the purified protein is correctly folded, and catalytic site of C-domain of angiotensin-converting enzyme possesses enzymatic activity and is inhibited by Lisinopril. This peptide can be used to test new inhibitors and C-domain of angiotensin-converting enzyme substrates because this peptide is easy to produce and this has proven efficient link with these molecules ("Structural characterization and enzymatic activity of the recombinant Ala959 to Ser1066 region of human ACE"). Osteoblasts are specialized fibroblasts that secrete and mineralize the bone matrix, and there is little information on the fate and potential therapeutic efficacy of low-dose gamma-irradiation in the formation of mineralization nodules in the osteoblast culture. Our first results of low dose irradiated osteoblasts showed an increase in the number of mineralized nodules.

Biological effects of ionizing radiation

Biological effects of ionizing radiation in aqueous solution, produces several highly reactive species. The most important are hydroxyl radical and hydrated electron. These products interact with peptides and proteins causing several modifications such as fragmentation, aggregation or oxidation, which are responsible for detoxification or even few modifications on proteins. These properties of ionizing radiation make it a good tool to improve antiserum production and vaccination process. Additionally, some substances called scavenger can be used to modulate these effects. It was found that the irradiated protein could be selectively incorporated to the cells, due to specific receptor for oxidized protein, the scavenger receptors. This increased uptake could also result in better antigen presentation and high immune response, either humoral, as demonstrated with purified crotoxin or cellular,

as recombinant M. leprae Rp 18 heat shock protein. Ionizing radiation can also modify biological and structural properties of toxins as crotamine, used here as a model. Biological and structural alterations occurred in irradiated crotamine were observed with spectroscopic assays, such as fluorescence, circular dichroism and mass spectrometry.

Animal Laboratory Division

The Animal Laboratory Division is a facility having 1040 m^2 of built area, distributed in production and stock areas of animal models for IPEN as well as for other institutions. Some of these models bred in this division are unique in Brazil, thus providing extremely useful tools for many investigators. The goal of this division is to act as an animal breeding and experimentation facility, sterilizing products and providing services to guarantee the genetic and sanitary quality of animals employed in investigations focusing mostly on the development of new drugs and radiopharmaceuticals (Fig. 5).

Besides breeding animals for use in our institution, this facility also sells animals for other laboratories and offers housing of special care requiring mice and rats upon request. For further information, contact bioterio@ipen.br.

Figure 5. Specific Pathogen Free animals kept under genetic, sanitary and environmental controlled conditions

Program Team

Research Staff

Dr. Carlos Roberto Jorge Soares, Dr. Cibele Nunes Peroni, Dr. Daniel Perez Vieira, Dr. João Ezequiel de Oliveira, Dr. Kayo Okazaki, Dr. Ligia Ely Morganti Ferreira Dias, Dr. Maria Helena Bellini Marumo, Dr. Marina Ferreira Lima, Dr. Nanci do Nascimento, Dr. Paolo Bartolini, Dr. Patrick Jack Spencer, Dr. Regina Affonso, Dr. Miriam Fussae Suzuki, M Sc. Cecília da Silva Ferreira, M. Sc. José Francisco da Silva Franco, Tech. Antonio Carlos Junqueira, Tech. Cicero Florêncio dos Santos, Tech. José Longino Ramos, Tech. José Maria de Souza, Tech. Manoel Calixto Lopes da Silva, Tech. Rosângela do Rócio Arkaten, Antonio Roberto Fernandes, Arlete Valéria de Souza Correia, Rute Marlene Batista

Postdoctoral Fellows

Dr. Cláudia Regina Cecchi, Dr. Cleide Falcone, Dr. Glaucie Jussilane Alves

Graduate Students

Amanda Ikegami Quinello, Andre Moreira Rocha, Asterio Souza Magalhaes Filho, Bruno Baessa Chargas, Caroline Cristina Elias, Cleide Mara Rosa da Silva, Cristiano Da Silva Granadier, Daniel Riani Gotardelo, David Peters dos Santos, Ed Carlos Santos E Silva, Eliana Rosa Lima Filha, Eliza Higuti, Elizabeth Brigagao de Faria Lainetti, Evelin Caroline da Silva, Felipe Douglas Silva, Fernanda Dos Santos Arthuso Perez, Fernando Jose Costa Baratéla, Flavia Albuquerque Magalhães, Flávia Gomes Silva Valgôde, Gabriela Ortega Coelho Thomazi, Gustavo Protasio Pacheco de Jesus, Ivette Zegarra Ocampo, Jose Pedro Prezotto Neto, Karina Corleto de Oliveira, Karine Alves Gonçalves Mota, Kleverson Wessel de Oliveira, Lea Rache Gaspar, Marcela Di Giacomo Messias, Marcio Martins de Araujo, Maria Ana Salviano de Sousa, Mariana D'angelo Martins Kmaid El-Corab, Nader Nazir Suleiman, Natan Versati da Silva, Patricia Marinho Sant'ana, Paula Juliana Nishimura, Paulo Victor Sarmento Dias, Raquel da Silva Aires, Renato Martins Araujo, Samuel de Brito Levindo, Tamara Mieco Fucase, Thais Cristina dos Anjos Sevilhano, Thompson de Oliveira Turibio

Undergraduate Students

Amanda Silva, Ana Carolina da Silva Cordeiro de Menezes, Ana Carolina Pedroso Romeiro Garcia, Andrea Von Gal Dainese, Ariane Cristina Miranda, Bruna Francine Harumi Uyeti, Camila Ayala Lira Da Cruz, Carolina Martim, Claudia Alves Dias, Danilo Barbosa Rocha, Eduardo Eriyo Hirai, Eliana Rosa Lima Filha, Emília da Silva Brandão, Evelin Caroline da Silva, Evily Fernandes da Silva, Fagner Sant'ana Januario, Felipe Marchi de Azevedo, Fernanda Mouro Galluzzi, Gabriela Oliveira Xavier, Giovana Maezano, Giuliana Improta Romano, Giuliana Mendonça Duarte Pinto, Ivette Zegarra Ocampo, Jenifer Pinheiro da Silva, Jessica Santiago Guimaraes, Josias Paulino Leal Silva, Larissa Dias de Souza, Leticia Emeterio Nascimento, Livia de Araujo Lima, Luiz Felipe Teixeira da Silva, Marcela Di Giacomo Messias, Marcela Pacheco de Almeida, Maria Neide Ferreira Mascarenhas, Marina Gordillo Fernandes,

Mayara Ledier de Azevedo, Millena Aparecida Sousa de Freitas, Nadja Felix da Silva, Natalia Mingorance Lombardi, Nathalia Mansur de Almeida, Paulo Henrique Souza Marcondes, Renan Passos Freire, Renata Ferreira Laranjeira Soares, Renato Ferreira de Castro, Sinday Pinheiro Alves, Suelen de Barros Sampaio, Thais Helena Lazari Pinto, Vitoria Maria Pereira, Viviane Santos Pereira

Collaborators

Dr. Álvaro Antonio A. de Queiroz, Dr. Fabiana Medeiros da Silva, Dr. Kayo Okazaki, Dr. Maria Teresa C. P. Ribela, Dr. Mariangela de Burgos Martins de Azevedo, Dr. Olga Zazuco Higa, Dr. Spero Penha Morato