

Balneotherapy and healthy ageing - review

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Abstract

“To be forever young doesn’t mean to be 20. It means to be optimist, to feel good, to have an ideal to fight for and to achieve it” said Prof. Ana Aslan. Human ageing and longevity are complex and multi-factorial traits that result from a combination of environmental, genetic, epigenetic and stochastic factors. Ageing refers to the time sequential deterioration - including weakness, susceptibility to disease, loss of mobility and agility.

INTRODUCTION

Ageing is a degenerative process, extensively studied for which many theories have been formulated. Ageing is the major biomedical challenge of our society, considered as a progressive and irreversible set of structural and functional changes for a living organism. The percentage of elderly people, the demographic imbalanced pyramids and the incidence of age-related diseases such as cardio-vascular diseases, cancer and neurodegenerative diseases are main concerns for many scientists.

There are 10 questions about ageing: 1) How does ageing and longevity vary between species?; 2) How does ageing evolve? 3) Are ageing and longevity controlled by the genome? 4) Can aging be suppressed and lifespan increased? 5) What is the basis of ageing and longevity? 6) How does cellular senescence contribute to ageing? 7) How does ageing give rise to ageing-related disease? 8) Why does the immune system fail in ageing? 9) What are the prospects for treatments for ageing? 10) What should the aims of ageing research be?

Healthy ageing should ideally start in childhood and take a lifelong perspective. Yet it is never too late to start. Investing in prevention can have important benefits for the individuals involved and has also societal benefits, since it is better to finance effective strategies to prevent

diseases than to use the resources to cure them [6].

Combining the balneotherapy with using products with healthy-ageing effect provides a significant advantage and represents the sustainability of the strategies for healthy ageing, in the context of which the spas is the ideal place for the application of new treatments. Darwin’s theory proposes survival and reproductive capacity as factors driving evolution. He assumed that all living organisms have the capacity for evolution, by means of natural selection, and that this capacity was a constant.

Richard Dawkins, author of “Selfish gene” says that genes are actually struggling for survival. Animals and humans are “survival machines” whose purpose is to propagate their genes. Evolvability theory refers to the idea that populations and species can vary in their capacity for evolution. Evolvability considerations provide explanations for programmed ageing and other lifespan limiting characteristics.

Ageing is intrinsically complex, being driven by multiple causal mechanisms, inclusive mutations. Each mechanism tends to be partially supported by data indicating that it has a role in the overall cellular and molecular pathways underlying the ageing process. Even in a population free of ageing, death will nonetheless occur, from extrinsic hazards as disease, predators, and accidents.

Ageing evolves as a side effect of natural selection in favour of mutations that cause a benefit during youth - The Pleiotropy or Trade-off Theory for the Evolution of Ageing. There are mutations that are beneficial in youth, but at the price of a higher rate of ageing. More individuals will survive to express the early benefit than will survive to suffer the higher rate of ageing.

Regarding The How of ageing question, one of the first theories is „The rate of living theory” – after which: „ the duration of life varies inversely as the energy expenditure, the length of life depends on the rate of living” as in the case of *Drosophyla*.

Unlocking the capacity to manipulate human ageing would result in great health benefits. Currently, health is understood as the removal of diseases in a defensive manner to the pathological process and with higher costs. Understanding how the environment modulates ageing-related genes may lead to human applications and disease therapies through diet, lifestyle, or pharmacological interventions. The study of the mechanisms by which various natural health factors can influence the ageing process opens the path to design and obtain new products for healthy ageing.

For the cellular level, Hayflick limit express that cell replication slows and stops after about 50 passages, becoming senescent.

Research on healthy ageing interventions has evolved along the main theories of ageing. Pharmacological intervention to decelerate ageing and age-related diseases is highly attractive. The potential for further advances in this field is immense; hundreds of genes in several pathways have recently emerged as regulators of ageing and caloric restriction.

One well-studied dietary manipulation of ageing is caloric restriction, which consists of restricting the food intake of organisms without triggering malnutrition. Caloric restriction is already being used as

a paradigm for developing compounds that mimic its life-extension effects with therapeutic value. Reducing calorie intake has been shown experimentally to induce anti-ageing behavior in many species.

Lifespan extension possibility was demonstrated for many geroprotector substances: Gerovital prepared by Ana Aslan, resveratrol, Rapamycin, antioxidants, hormones, bioregulatory peptides, metformin, fenformin and adaptogens (ginseng). As mechanisms have been observed reduced plasma levels of insulin, increased insulin receptor sensitivity, low body temperature, cholesterol, triglycerides, alpha-lipoproteins, blood pressure. All these processes are correlated with the expression of genes called sirtuins. Rapamycin, described as an immunosuppressive drug has been shown to be also an anti-ageing drug whose goal is the mTOR - a serine/threonine kinase that regulates cell growth, viability, mobility, protein synthesis and gene transcription.

Among the many hormones with geroprotector effects, estrogen-like or testosterone-like substances, detected also in the organic component of therapeutical peloids, stimulate or inhibit various neuroendocrine mechanisms. If insulin and cortisol serum levels are increasing with age, the testosterone, estrogen, progesterone, growth hormone and thyroid hormones level gradually decrease along with age, which causes progressive muscle atrophy, fatigue, osteoporosis, reduced sexual function and increased fat levels.

Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. Reactive oxygen species have been found to play an important role in the initiation or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease. An imbalance between antioxidants and reactive oxygen species results in oxidative

stress, leading to cellular damage. Air pollutant such as car exhaust, cigarette smoke, and industrial contaminants constitute major sources of ROS that attack and damage the organism.

Herbs, diet, lifestyle, and supplements can slow ageing, enhance memory, help prevent cancer and heart disease. Medicinal plants (many used as spices and food plants) contain organic compounds as tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids, which produce physiological effects. Antioxidants intervene in gerossuppression based on the ageing free radical theory. Resveratrol present in grapes and subsequently in red wine, is a natural phytoalexin used by certain plants in the defense mechanism against pathogens. Resveratrol caused a statistically significant decrease of age-related parameters such as albuminuria, inflammatory level, vascular endothelial apoptosis, and the incidence of cataract, at rats. A healthy diet should provide the different nutrients one needs to remain healthy and should give the opportunity to engage socially and to have a good quality of life. However, the precise dietary needs of the older people are still largely unknown.

Peloids used also in the treatment of various rheumatic, endocrine, dermatological or gynecological diseases, represent the support for the design of new geroprotectors. "Peloidextract" obtained from Techirghiol mud is a clear liquid, with an alkaline pH of 7.6 to 8, and mineral content closely comparable to that of blood serum. Humic compounds play a role in redox reactions, absorption, complexation and transport of substances. Humic acids have an astringent effect, anti-allergic, antiviral, antibacterial, anti-inflammatory, estrogenic, hyperemic, UV-protective and are heavy metal chelating-agents. Fulvic acids have antitumoral, anti-allergic, antioxidant and antimicrobial activity, acting in acid medium by inhibiting mitochondrial respiration in

Candida utilis, have antiulcerogenic and precognitive properties so they can be used to treat Alzheimer's disease.

Theories of ageing

While earlier theories of ageing were centered on the level of organ dysfunction or systemic level of the body, current theories put focus on dysfunction of the cellular and molecular mechanisms [10]. Such modern approaches reduced the differences between the existing theories on the mechanism of ageing, emphasizing their complementary elements, but at the moment there no integrative theory [2] exists for post-reproductive ontogenetic development that can explain all the processes involved in the multiple aspects of ageing.

Programmed ageing theories are subdivided into three conceptual subcategories [2,13,14] genetically programmed longevity theory which assumes that ageing is the result of starting or stopping certain genes, being included the role of genetic instability (shortening of telomeres) in the dynamics of ageing processes [14, 17]; endocrine theory - according to which ageing is governed by a biological clock, whose function is regulated by endocrine mechanisms, including insulin-like growth hormone IGF-1 [2,3,14]; and immunity theory, which states that the immune system can be programmed to a decrease in its functionality, immunosenescence, which will lead to increased susceptibility to infectious diseases and ageing [15,16,25,26].

Theories which put the ageing process due to internal and external environmental factors [10] are *Wear and Tear Ageing Theory* according to which cells and tissues become aged over time [2,14]; *vital level activity theory* - which states that a high level basal metabolism leads to a shortening of the life of the organism [2,14]; *cross-linking theory* presented by Bjorksten (1942) according to which the

cumulative chemical interchange of important macromolecules, including collagen, causes ageing [2,14]; somatic DNA damage theory [47] which considers that ageing results from genetic integrity degradation of somatic cell as a result of mutations registered both in the nucleus and in the mitochondria [2,13,14,44]; and free radicals theory which stipulates that the superoxide and other free radicals destroy the molecular components of cells and thus alter their normal function [11].

Considering ageing being a gradual loss of functional adjustment of some complex multifactorial biological processes, the individual genotype surely has an impact on the rate of ageing [11,12,18]. More recent studies also include metabolomic approaches towards identification of metabolite panels and potential metabolite markers indicative of age-related processes, and include e.g. amino acids, fatty acids and steroids, branched-chain amino acids and polyamines.

In 1957 Harman proposed the idea that a general process of accumulation of oxygen-free radicals negatively affects internal environmental factors and changes genetic factors, mechanism responsible for ageing and death of all living things. The theory was revised in 1972 when it was shown that mitochondria are the main center of several chemical reactions that generate free radicals. Reactive oxygen species (ROS) are probably the most significant free radicals with major implications in damage and cells and body ageing [19,20]. Free radical theory is currently considered the most acceptable explanation of ageing [19], although recent data obtained on a set of transgenic mutant $SOD2^{+/-}$ or $Melk1^{+/-}$ mice disprove the central dogma of the theory [11].

The main bottleneck is the characterization of the rate of ageing of the body, which employs the concept of biomarker of the ageing [21,24,27], defined as quantitative or qualitative biological parameter that allows the

prediction of functional status of the organism to a particular moment of its existence [22,24]. Experts in the field suggest parameters that aim the age of nervous, cardiovascular, respiratory or excretory system. Also, they are advancing proposals for estimating the biological age of the cell. In humans, the biological age is surpassed by the psychological age, the intellectual age and the social age.

Several authors have discussed the criteria for selection of some biomarkers of ageing, emphasizing the following critical points that they should fulfill [23]: ageing and longevity rate prediction, quantitative monitoring the biological quality of the body by parameters by which the determination will not harm the integrity of the organism, the data obtained must be reproducible and comparable for different species of laboratory animals and with relevance for humans [23,25,27,28]. Among the main biological markers used to predict the biological age and longevity, the American Federation of Ageing Research suggests as the set of biomarkers of ageing [27]: systolic and diastolic blood pressure, the heart rate at rest, total cholesterol, HDL and LDL cholesterol, lipid levels, blood glucose, body mass index [26], abdominal obesity index, T lymphocytes level, cortisol level and electrocardiogram.

Allostatic oversteering index [23] proposed by McEwen and Stellar in 1993 denotes the body's dysfunctional response to chronic stress and reveals increased levels of adrenaline, noradrenaline and dopamine hormones [2]. This index was used by Karlamangla and collaborators in a recent study of mortality in people aged over 70 years, pursued over a period of 4.5 years. Patients with an allostatic oversteering increased index presented a much higher risk of mortality. Thus, the study concludes that a uniform increase of allostatic oversteering index (normally located between -1.7 and 1.4) leads to an increase of 3.3 times in mortality measured in the range of 4.5 years.

The so-called **matrix protocol** [2] of ageing biomarkers used by the International Institute of Longevity Montclair (New Jersey) determines the rate of ageing on four levels:

I. the overall functionality of the body measured by the ratio of weight and level of fat, flexibility, aerobic endurance, bone density, tactile response latency, forced expiration volume, vision and hearing [27];

II. functioning at the cellular level monitored at the skin level by determining the cytoarchitecture changes of basal membrane, epidermal turn-over, sebaceous glands architecture, microvascular changes, collagen ratio, elasticity of the fibers [28];

III. the molecular level consider the growth and thyroid hormone level but also of coenzyme Q10, sensitivity to insulin, the heat shock proteins level, the oncogenes level in the blood, serum level of antioxidants [25];

IV. at DNA level is studied the position and size of telomeres (set at WBC level) and the accumulation level of mutations in the genetic material.

The researchers from the International Longevity Institute are currently working on a new laboratory chemistry test that should reflect the damage level of DNA and should bring science-based predictions regarding the therapeutic value of anti-ageing interventions through the mutational changes occurring in the somatic genetic material [2, 28, 29].

Telomeres damage can be assessed indirectly by measuring some biomarkers that correlate with their shortening [28]. Thus, Jiang and Rudolph analyzed the telomeres shortening mechanism and the rate of ageing in several organs and tissues, identifying four proteins whose expression increases in relation to telomere shortening, as these: Cathelicidins from macrophages and polymorphonuclear leukocyte lysosomes activated by bacterial infection; chitinase 3 like3 (Chi 3L3) involved in the initiation of immune

response; elongation factor 1 α (EF-1 α) - which controls protein synthesis in human fibroblasts and stathmins - which control the intracellular microtubules stability, cell motility and mitosis [2].

The concept of oxidative stress caused by free radicals, represents the basis of taking into account biomarkers of ageing, physiological and molecular indicators of oxidative stress. Carbonylate proteins represent, in this context, an example of the use of oxidative stress indicators as biomarkers of ageing [2,25]. Many scientists consider the accumulation of protein carbonyls in erythrocyte membrane as an indirect marker of ageing and the ferro-reduction antioxidant potential (FRAP) in blood plasma as an indicator of the body's antioxidant defense capacity [2]. The oxidative and reductive activity of enzymes that act on glutathione, thioredoxin and other substrates of interest in the oxidation-reduction process reflects not only the level of antioxidative protection but are probably relevant biomarkers for ageing rate. NADH and FADH coenzymes - critical components of energetic transport in mitochondria may represent, by their expression level, biomarkers of mitochondrial activity and indirectly of senescence and body age [2].

Heat shock proteins (HSP) are other biomarkers of ageing, and there are studies showing that the change of these proteins expression correlates with changes in longevity, experimentally described in *Caenorhabditis elegans* [2].

Apolipoproteins synthesized in the liver and intestine are involved in the transport of cholesterol and triglycerides in the blood, are considered more eloquent predictors of death by coronary heart disease [23].

Interleukin 6 (IL6) is a multifunctional cytokine that plays an essential role in acute inflammation stage. Although normally in the absence of inflammation can not be detected in blood samples over time by losing his regulation expression,

the expression level increases with age [22,23].

Growth hormone, secreted by the pituitary gland [351] has a decreasing level with age of the body being registered a decrease by 14% every decade after the age of 20-25 years and reaches half around the age of 60 [2, 21,22,26].

Geroprotectors

Lifespan extension possibility was experimentally demonstrated for many geroprotector substances, including antioxidants [75], latirogene, chelating agents (carnosine [53]), adaptogens, neurotropes, monoamine oxidase inhibitors, dehydroepiandrosterone, sexual hormones, growth hormone, melatonin [50], preparations from the pineal gland, protease inhibitors, antidiabetic agents, thymic hormones, immunomodulators, superoxide dismutase and catalase mimetics, enterosorbents, etc. [47].

There are not yet geroprotectors with unquestioned efficiency, and in some cases, supposed geroprotector substances had proved long term effects difficult to predict initially [2, 79].

In the past years, a new area of the gerontology becomes important, that of studying the so called *hormesis* mechanisms by which substances classified as toxic or very harmful, in subliminal doses, may have geroprotector effects; currently numerous scientific investigations are undertaken in this regard [2].

Reducing calorie intake [177, 192] has been shown experimentally to induce certain anti-ageing behavior in a variety of species [31,32,33,58]. But for humans and other long-living animals, reducing calorie intake interferes with many other genetic parameters and diet composition.

Studying the mechanisms by which calorie intake reduction influences senescence and longevity rate in experimental animals [82], researchers have correlated this required behavior with

reduced plasma levels of insulin [81], increased insulin receptor sensitivity, low body temperature, cholesterol, triglycerides, alpha-lipoproteins, blood pressure and an increase in HDL cholesterol. Setting all these processes is experimentally correlated with the expression of certain genes called sirtuins [96] found in a wide range of organisms from yeast to humans [39].

In 2011 there have been globally described 30 substances with geroprotector properties [2, 288], along side Gerovital - Romanian geroprotector product prepared by Prof. Ana Aslan in 1951, based on anesthetic procaine, noteworthy resveratrol and other polyphenols of plant origin [30], Rapamycin, antioxidants (vitamins A, C and E, carotenoids, lipoic acid, coenzyme Q, selenium, etc.), hormones (GH, thyroid hormones and sexual hormones, melatonin [50]), bioregulatory peptides (timalin, epithalmin), biguanides (metformin, fenformin) [77], and adaptogens (ginseng [48]).

Scientific reports show that biguanides, particularly metformin [56-77], used in the treatment of type 2 diabetes due to their ability to inhibit gluconeogenesis and to determine the increase of sensitivity to insulin, may also have geroprotective effects [56, 98].

Sirtuins are NAD⁺-dependent protein deacetylases regulating metabolism and ageing processes, and they were suggested to mediate lifespan-extending effects of caloric restriction. They were discovered in *Saccharomyces cerevisiae*, and have attracted interest due to their involvement in lifespan extension, age-related disorders, obesity, heart disease, neurological function and cancer [36]. In mammals sirtuins family consists of seven genes of this type, called Sirt1 to Sirt7 [35]. Recent researches seek to identify their role in cell physiology and mechanisms or molecular agents, and ways of how their activity can be influenced [31, 32, 35,105].

Sirolimus or rapamycin, described as immunosuppressive and antitumoral drug

has been shown to be also an anti-ageing drug [41] whose goal is the TOR gene in yeast [43] and mTOR gene, its mammalian correspondent, also known as FK506 factor or FRAP1, a serine/threonine kinase that regulates cell growth, viability, mobility, protein synthesis and gene transcription [31,45,78]. Rapamycin, according to recent studies, may act preventively in atherosclerosis, hypertonia and hypercoagulation, thus preventing myocardial infarction, cardiovascular accidents, osteoporosis, cancer, autoimmune diseases, arthritis, fat diabetes, Alzheimer and Parkinson diseases [31,42,45].

Antioxidants such as α -tocopherol, ascorbic acid, retinol, ubiquinone, selenium, as endogenous compounds or numerous synthetic molecules, intervene in gerossuppression based on the ageing free radical theory [87]. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is the best known current geroprotector, discovered in 2003, present in high concentrations in the epicarp, seeds and stalk of grapes (*Vitis vinifera*) and subsequently in red wine [30,33], and in roots of *Polygonum cuspidatum*. It is a natural phytoalexin used by certain plants in defense mechanism against pathogens such as bacteria and fungi, currently extensively studied, and that can influence the expression of sirtuins [34,35,37,102]. Since its discovery in 2003 until today there were registered worldwide over 2,000 experimental studies on resveratrol, and several laboratories dedicate their work to the study of this substance [2].

Preclinical studies [103] on resveratrol demonstrated an increase in the longevity of *S. cerevisiae* of 70% by cultivation in a medium containing 10 mM resveratrol, of 20% in *C. elegans* and 29% in *Drosophila melanogaster* by treatment with 100 mM resveratrol [39,40]. In studies on laboratory mice, resveratrol at doses around 20 mg/kg caused a statistically significant decrease of age-related parameters such as albuminuria, the inflammatory level,

vascular endothelial apoptosis, decreases in the elasticity of the aorta, the incidence of cataract, etc., being inclusively registered data towards the decrease of genetic instability [2].

On MRC5 human fibroblasts, the resveratrol in concentration of 5 μ m induced a significant protection of DNA oxidative deterioration, preventing the growth of nuclear volume, reducing the generation of acetylated forms of H3 and H4 histones and p53 protein. In another study on human fibroblasts, using a concentration of 10 μ m and 25 μ m have been obtained data supporting the delay in the morphological alterations at the cellular level correlated with age [2].

It is supposed that polyphenols such as resveratrol inhibits senescence at the cellular level by activating genes such as p53 and AKT [39], sirtuins or inhibition of others, like mTOR [46]. These influence different intracellular signaling pathways by which are controlled the expression of genes involved in cell growth, the proliferation and cell viability [34]. Clinical trials regarding resveratrol action in oncology that used a commercial form of resveratrol called SRT501 showed a 39% increase in apoptosis of malignant cells in patients with metastatic colorectal cancer [2, 194, 195]. Neuroprotective effects of resveratrol [30,35] have been described in experimental studies on laboratory mice and were explained by researchers by the effect of resveratrol of increasing the cysteine level that can protect cells from oxidative stress by regulating proteic precursors of amylase plate [38]. Resveratrol acts also on manganese superoxide dismutase (MnSOD) a group of enzymes that degrades metabolic generated superoxide molecules thus having antioxidant effect [80].

Cardioprotective effects of resveratrol [30,36], as well as the other polyphenolic antioxidants such as quercetin or catechins, was observed in *in vitro* studies that showed a reduction of cardiomyocyte

apoptosis by decreasing the level of caspase-3 and other cytokines, including NF- κ B, E-selectin, TNF- α or troponin [2, 224].

Resveratrol also has an anti-inflammatory effect [30,33], resulting in decreased activity of cyclo-oxygenase with a key role in the synthesis of other cytokines such as IL17. Resveratrol acts on the cellular mechanisms involved in photo-ageing correlated with UV action, including MAP kinases, NF- κ B nuclear factor and matrix metalloproteins. External applications of resveratrol in SKH-1 hairless mice model prior to UV exposure caused a significant reduction of cell proliferation, protection mRNA and phosphorylation [30]. The theory of using antidiabetics like resveratrol [30] is explained by indirectly activating SIRT1 and subsequent increase in sensitivity to insulin [32], improving microcirculation and peripheral nerve function [2]. The pharmacology of resveratrol [34] is still marked by a number of limitations, having a low solubility in water (only 0,03g/L) and consistently a low bioavailability and stability, being easily oxidized in the presence of light or heat. There are also data of some authors that contradict the fact that resveratrol would determine the extent of longevity [280], obtained on *Drosophila melanogaster* and *C. elegans* [36].

Among the many hormones above mentioned with geroprotector effects [49], greater relevance for this project have the substances with similar actions of sexual hormones. Substances such estrogen-like, testosterone-like, which stimulate or inhibit various neuroendocrine mechanisms [49] are detected in the organic component of therapeutical peloids and their geroprotective action is currently intensively studied. The testosterone serum level gradually decreases along with the advancing in age which causes progressive muscle atrophy, fatigue, osteoporosis, reduced sexual function and increased fat levels. These symptoms can be treated by

administration of testosterone [22], which has as effect, in addition to a recovery of lipid levels, also a decrease of concentration of plasma glucose correlated with increased sensitivity to insulin. The last half of the century allowed due to numerous scientific results, the use of estrogen in postmenopausal women to prevent signs of ageing, such as osteoporosis, cardiovascular diseases, decrease sexual and cognitive functions [52]. However, the enthusiasm was diminished by recent publications indicating the risks of using estrogen, including breast cancer, pulmonary embolism, stroke and coronary heart disease [2, 55].

Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function [277]. Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species (ROS) in the cells. Free radicals can initiate the oxidation of bio-molecules, such as protein, lipid, amino acids and DNA, which will lead to cell injury and can induce numerous diseases. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress is the main cause of several diseases: cancer, cataracts, age-related diseases and Parkinson's disease [208, 214, 222, 225]. Air pollutant such as car exhaust, cigarette smoke, and industrial contaminants encompassing many types of NO derivatives constitute major sources of ROS that attack and damage the organism either by direct interaction with skin or following inhalation into the lung [83]. Drugs are also a major source of ROS [84,85]. One of the major sources of oxidants is food, which often contains different kinds of oxidants such as peroxides, aldehydes, oxidized fatty acids, and reactive species catalysts (transitional metals) [86]. Food debris that reaches the

intestinal tract places an enormous oxidative pressure on the intestinal-tract mucosa [89]. Although the exposure of the organism to ROS is extremely high from exogenous sources, the exposure to endogenous sources is much more important and extensive, because it is a continuous process during the life span of the organism [90].

Enzymes comprise another endogenous source of ROS. While most enzymes produce ROS as a by-product of their activity, exemplified by the formation of superoxide radicals by xanthine oxidase, there are some enzymes designed to produce ROS, such as nitric oxide synthase that yields NO radicals, those that produce H_2O_2 , and those responsible for hydroxylation [93]. Blood cells, except erythrocytes (neutrophils, eosinophils, basophils, monocytes and lymphocytes), possess mechanisms to combat bacteria and other invaders by ROS production that act synergistically with exogenous ROS and NADPH serves as a donor of electrons to an activated enzymatic complex in the plasma membrane (NADPH-oxidase complex) to produce superoxide radicals from the oxygen molecule. Following dismutation, the production of H_2O_2 leads to the formation of OH^\bullet by the metal-mediated, Haber-Weiss reaction [51].

Molecular oxygen is required to sustain life, but it can be toxic through the formation of reactive oxygen species (ROS). ROS includes superoxide radical, hydroxyl radical, singlet oxygen and H_2O_2 , which have been found to play an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease [209].

In several normal conditions ROS are produced and play a role in the pathogenesis of the physiological condition. Literature data show that numerous pathologies and disease states serve as sources for the continuous production of ROS and more than 200 clinical disorders have been described in

which ROS (as initiators or as mediators) were important for the initiation stage of a disease or produced during its course (e.g. cancer, heart diseases, endothelial dysfunction, atherosclerosis and other cardiovascular disorders, inflammation, burns, intestinal tract diseases, brain degenerative impairments, diabetes, eye diseases, and ischemic and postischemic pathologies [94]. Other pathological disorders, which are associated with impaired metal metabolism, such as hemochromatosis, Wilson disease and thalassemia, in which iron is deposited in many organs, are known to increase significantly the concentration of ROS. Also, these are exemplified during the aging process where ROS production significantly increases as a result of impaired mitochondrial function and in the early stages of embryonic development [51].

Organisms have multiple mechanisms to protect cellular molecules (DNA, RNA and proteins) against ROS-induced damage. These include repair enzymes (DNA glycosylases, AP endonucleases etc), antioxidant enzymes (SOD, catalase, and glutathione peroxidase), and intra as well as extracellular antioxidants (glutathione, uric acid, ergothioneine, vitamin E, vitamin C and phenolic compounds). However, this natural antioxidant mechanism can be inefficient for severe and/or continued oxidative stress [209].

Nutritional deficiencies, impaired digestion, poor circulation, insulin resistance, smoking, and chronic stress all can contribute to diminished cerebral function and may be part of the Alzheimer's puzzle. No one has discovered Ponce de Leon's Fountain of Youth, but we do know that herbs, diet, lifestyle, and supplements can slow ageing, enhance memory, help prevent cancer and heart disease, and keep us as healthy as possible throughout our lives. In this project we will focus on common conditions associated with ageing and examine a range of herbal

and nutritional therapies that can help [216].

Herbal formulations have been in use for many years globally not only as therapeutic but also as prophylactic and health promotive agents. Sea buckthorn (*Hippophae rhamnoides* L.), a unique and valuable plant has recently gained worldwide attention, mainly for its medicinal and nutritional potential. Sea buckthorn is a thorny nitrogen-fixing deciduous shrub of cold arid region native to Europe and Asia. It is currently domesticated in several parts of the world due to its nutritional and medicinal properties. All parts of this plant are considered to be a good source of large number of bioactive substances like vitamins (A, C, E, K, riboflavin, folic acid), carotenoids, phytosterols (ergosterol, stigmasterol, lanosterol, amyryns), organic acids (malic acid, oxalic acid), polyunsaturated fatty acids and some essential amino acids [109, 168, 169, 172, 243, 223, 227].

Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. This activity is due to the ability of antioxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation. Medicinal plants contain some organic compounds, which produce definite physiological action on the human body, and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. They are of great importance to the health of individuals and communities. Many of these indigenous medicinal plants are used as spices and food plants [208,210,218,222,229].

Phenolics have been known to possess a capacity to scavenge free radicals. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors. Phenolics are

especially common in leaves, flowering tissues and woody parts, such as stems and barks [208, 211, 212,220]. Although it is still early to define their exact clinical benefits for treating age-related disease, a diet rich in polyphenolic or other forms of antioxidants does seem to offer hope in delaying the onset of age-related disorders. It is now clear that any deficiency in antioxidant vitamins, inadequate enzymatic antioxidant defenses can be distinctive for many age-related disease, and protein carbonylation can be used as an indicator of oxidative stress associated to diseases and ageing status [213].

Mud and healthy ageing

The liquid phase of mud constitutes a hypertonic solution of minerals, organic humic substances, bituminous, carbohydrates, peptones, amino acids and enzymes. Since 1957 V.D. Narti proposed the utilization of natural solutions of this therapeutic mud, besides classical procedures with mud. The solution envisaged by Narti, "PELOIDEXTRACT", was extracted by filtration under pressure of 6-12 atmospheres of inert gas (CO₂). The solution obtained from Techirghiol mud is a clear liquid, stable, with a density of 1.062 at 21°C, with an alkaline pH of 7.6 to 8, and mineral content closely comparable to that of blood serum, except SO₄²⁻ and Mg²⁺, which are in excess. The natural solution would be a hypertonic solution 10 times more concentrated than blood serum.

For cellulite treatment extracted mud is mixed to form a cream with plant extracts and bioactive substances [120, 215, 217]. In psoriasis, seborrheic and atopic dermatitis, eczema and first degree burns is used a cream containing 1-6% suspension of mud as active ingredient [121]. In addition, the mud suspension may be used to create dermal application dressings [122, 123]. The mud is composed of humic substances, pectin, cellulose and lignin, waxes, resins and inorganic materials

[124], also including identified structures such as alkanes, 4-phenyl valeric acid, 5-isopentyl picolinic acid, 33hydroxylauric acid, (5 α , 3 β)-3-hydroxy-11-androstanon, 5 α -2-ene-11-androstenone, squalene, α terpineol, menthol derivates, palmitic, oleic and eicosanoic acid and the isoprenoid phytan [125].

Patent No116867B since 1996 requested by the SC "BIOTEHNOS" SA Bucharest describes a peloid-extract, which is based on sapropelic mud from Techirghiol Lake, characterized as a slightly-yellow colored liquid, slightly opalescent, odorless, with total salt: 116.4 to 118.3 g/l, mineral residue 75 to 80 g/l, Na⁺ from 24.3 to 25.0 g/l, K⁺ 0.7 to 0.9 g/l, Ca²⁺ from 0.08 to 0.19 g/L, Mg²⁺ 2.9 to 3.4 g/L, HCO₃⁻ 0.2 to 0.65 g/L, Cl⁻ 39.4-41.4 g/l, SO₄²⁻ 7.1-10.9 g/l, carbohydrates, lipids and proteins detection limit level and anti-hyaluronidase activity of minimum 30UI/ml. The peloid extract is in the form of a white powder, amorphous, with anti-inflammatory, antiseptic and anticongestive properties having a pH between 7.6 and 7.8, a rH of 8.2 and a density of 2.011, composed of 53.18% water-insoluble minerals which contain 0.2% magnesium silicate, 0.54%calcium silicate, 0.03% iron silicate and 1.74% aluminium silicate. Pharmaceuticals used in cosmetics or in the treatment of various pathologies is based on a total extract of mud, sometimes being fractionated. Thus, there are cosmetic preparations for which the active compounds from the mud are extracted using mixtures of paraffin/ethanol/dimethicone or a mixture of water/ethanol, or one containing water/ethanol / glycerol/propylene glycol [119].

Humic compounds play a role in redox reactions, absorption, complexation and transport of substances, supporting structure and formation of mud and control the carbon biogeochemistry in ecosystems [126]. Humic compounds can be classified into three fractions based on solubility in water at different pH values. Humic acid is

soluble in alkaline solutions fraction, fulvic acid is soluble in water fraction regardless of pH and the humine fraction is insoluble in water [127,128]. Humic acids are compounds having a molecular weight between 10,000 and 100,000 with structures containing aromatic ring structures with nitrogen atoms, aliphatic and peptide residues.

Humic acid [257, 310] containing 56% C, 5.5% H, 4% N and 33% O, the major functional groups present being COOH-, OH- phenol and ketone groups [129]. Humic acids have an astringent effect [352], adrenaline and dopamine receptor agonist, anti-allergic, antibacterial, anticoagulant, anti-inflammatory, antiviral, estrogen, hemostatic, hyperemic, UVB-protective [123, 342] and are heavy metal chelating-agents [129,130,131]. In rats treated with natural mud or mud humic acids extracted from mud, a decrease in total cholesterol, total lipids and blood glucose and increased LDH, immunoglobulins, erythrocytes, hemoglobin and hematocrit was observed [132, 297]. Humic acids can be extracted and purified using the method based on pyrophosphate- sodium hydroxide [128,133,134,137] or ionic liquids such as dimethyl ammonium dimethylcarbamate and 1-butyl-3-methylimidazole chloride [136].

Fulvic acids are oxidized substances with aromatic structures characterized by extensive lateral aliphatic chains having a lower nitrogen content compared to humic acids [127, 336]. Fulvic acids have anti-allergic effect [137,138], show antioxidant [139] and antimicrobial activity [140, 284], reduce cutaneous immune response [141], are antitumoral [142] and antiseptic [143], acting in acid medium by inhibiting mitochondrial respiration in *Candida utilis* [144], being used in the treatment of eczema [145], have antiulcerogenic properties [146] and precognitive so they can be used to treat Alzheimer's disease [147], not having adverse effects on the body in small doses [148]. According to

the method of the International Humic Substances Society [149] fulvic acids are separated from the acidified supernatant of an alkaline extract of mud using polymethyl methacrylate-based resins DAX-8 [150] or XAD-8 [151] based on hydrophobic interactions between fulvic acids and these resins. Other ion exchangers are used for the separation of fulvic acids are diethylaminoethyl cellulose and polyvinylpyrrolidone [152, 302]. Also, fulvic acids are separated by ion pair formation with cationic surfactants such as cetyltrimethylammonium benzildimethylhexadecylammonium bromide, dodecylethyldimethylammonium chloride [153].

The humic substances have functional groups available for the binding of metallic elements [110] such as Cu^{2+} [108], Cd^{2+} [107,106], and also transuranic elements (Pu and Am) [100]. A study of stability constant indicated that Am(III) had the strong interaction with humic acid, [99] and fulvic acids interact with Cu^{2+} , Zn^{2+} , Mn^{2+} [472] and U^{4+} or UO_2^{2+} [101].

Lipid fraction [219, 328] of the mud is defined as organic material insoluble in water and can be solubilized and extracted using apolar organic solvents such as hexane, benzene, chloroform, and diethyl ether [154].

This ratio is of 0.2 to 5% of the mud and include fatty acids [219], sterols, terpenes, hydrocarbons, chlorophyll, fats, waxes and resins [155]. The lipids from mud can be extracted by Soxhlet method using a mixture of benzene/methanol [156] or dichloromethane/methanol [157] or by the Bligh-Dyer method with a mixture of chloroform / methanol [158]. Another fraction of mud being of therapeutic interest consists of protein hydrolysates, amino acids, enzymes (amylase (EC 3.2.1), arylsulfatase (EC 3.1.6.1), β -glucosidase, cellulase (EC 3.2.1.4), chitinase, dehydrogenase, phosphatase (EC 3.1.3), protease, urease) and carbohydrates [159], which are released from the complex with humic substances by acid hydrolysis [160]

and concentrated by reverse osmosis [161 162].

Combining the best fractions of mud [358] obtained will result in getting the mud extract that will be used to obtain the new healthy-ageing products by adding plant extracts in a xylitol matrix. Xylitol is a five carbon sugar alcohol that naturally occur in foods such as fibrous vegetables and fruit. It is a natural, intermediate product which regularly occurs in the glucose metabolism. Xylitol is produced naturally in our bodies; in fact, we make up to 15 grams daily during normal metabolism. Xylitol has 40% fewer calories than sugar [54].

All the above data about life extension, geroprotectors, peloid and plant components, antioxidants, diet elements and phytochemicals constitute the general scientific background from which the proposal goes on, looking to combine selected fractions of peloid and plants in new healthy ageing products and to test them on animals, cells and humans [226].

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