

*EDITORIAL***PRESBYOPIA OPTOMETRY METHOD BASED ON DIOPTER REGULATION AND CHARGE COUPLE DEVICE IMAGING TECHNOLOGY**

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With the development of photoelectric technology and single-chip microcomputer technology, objective optometry, also known as automatic optometry, is becoming precise. This paper proposed a presbyopia optometry method based on diopter regulation and Charge Couple Device (CCD) imaging technology and, in the meantime, designed a light path that could measure the system. This method projects a test figure to the eye ground and then the reflected image from the eye ground is detected by CCD. The image is then automatically identified by computer and the far point and near point diopters are determined to calculate lens parameter. This is a fully automatic objective optometry method which eliminates subjective factors of the tested subject. Furthermore, it can acquire the lens parameter of presbyopia accurately and quickly and can be used to measure the lens parameter of hyperopia, myopia and astigmatism.

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PHYSIOPATHOLOGY OF OSTEOPOROSIS: FROM RISK FACTORS ANALYSIS TO TREATMENT

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Osteoporosis represents a relevant health issue, being the first cause of bone fractures in the elderly with subsequent implications in terms of survival and social costs. The improved knowledge about the physiopathology of this disease has led to a new definition of Osteoporosis, which shifts the attention from the “decrease in bone mass” to several elements related to what has globally been defined as bone quality. In fact, it has been shown that clinical risk factors affecting bone homeostasis coincide with osteoporosis risk factors. The evaluation of such clinical risk factors is an important element in the assessment of the global fracture risk. The availability of instruments for the assessment of the global fracture risk also suggests a change in the clinical perspective and raises new questions as yet unanswered.

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EDITORIAL

CLINICAL APPLICATION OF SHOCK WAVE THERAPY IN MUSCULOSKELETAL DISORDERS: PART IR. SAGGINI¹, A. DI STEFANO², A. SAGGINI³ and R.G. BELLOMO⁴

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The shock wave has been widely recognized in literature as a biological regulator; therefore we carried out a review on the activity performed by shock waves on the bone-myofascial tissue system. To date, the application of Shock Wave Therapy (SWT) in musculoskeletal disorders has been primarily used in the treatment of tendinopathies (proximal plantar fasciopathy, lateral elbow tendinopathy, calcific tendinopathy of the shoulder, and patellar tendinopathy, etc.) and bone defects (delayed- and non-union of bone fractures, avascular necrosis of femoral head, etc.). Although the mechanism of their therapeutic effects is still unknown, the majority of published papers have shown positive and beneficial effects of using SWT as a treatment for musculoskeletal disorders, with a success rate ranging from 65 to 91%, while the complications are low or negligible. The purpose of this paper is to inform the reader about the published data on the clinical application of SWT in the treatment of musculoskeletal disorders. In this paper, with the help of a literature review, indications and success rates for SWT in the treatment of musculoskeletal disorders are outlined, while adequate SWT parameters (e.g., rate of impulses, energy flux density, etc.) are defined according to the present state of knowledge. Given the abundance of the argument, it seems appropriate to subdivide the review into two parts, the first concerning the evidence of Extracorporeal Shock Wave Therapy (ESWT) on bone disorders, the second concerning findings on tendon and muscle treatment.

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MANAGEMENT OF PSORIATIC ARTHRITIS: SHOULD THE INTERACTION BETWEEN DERMATOLOGISTS AND RHEUMATOLOGISTS IN CLINICAL PRACTICE BE INTENSIFIED?

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Psoriatic arthritis is an inflammatory seronegative spondyloarthropathy that occurs in approximately 25% of patients with psoriasis and is a progressive and severely disabling disease. Most patients have had psoriasis for several years before the development of arthritis or develop the joint and skin condition simultaneously. Given the absence of specific diagnostic test for psoriatic arthritis, clinical findings remain the standard criteria for the diagnosis. Patients with psoriasis presenting to the dermatologist for management of their skin disease may have joint symptoms related or not to psoriatic arthritis and similarly arthritic patients presenting to a rheumatologist for the management of psoriatic arthritis may have skin lesions related or not to psoriasis. In this paper an expert panel of specialist belonging to the “Associazione Dermatologi Ospedalieri Italiani” (ADOI) and “Collegio dei Reumatologi Ospedalieri Italiani” (CROI) analysed the international literature and scientific recommendations and also investigated the Italian setting. It has been demonstrated in the literature that a multidisciplinary clinical setting may benefit patients with psoriatic arthritis from both diagnostic and therapeutic points of view. The comparative analysis of the Italian clinical records used by ADOI and CROI have highlighted some substantial differences. Collaboration between the dermatologist and rheumatologist allows for a more complete appreciation of the overall skin and musculoskeletal disease burden, and subsequently leads to a more comprehensive treatment approach.

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EDITORIAL

IMMUNOMODULATORY EFFECTS OF VITAMIN D ON SKIN INFLAMMATION

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Vitamin D has a major role in calcium absorption and maintenance of healthy bones. Vitamin D is also involved in cancer, cardiovascular system, allergic diseases, immune regulation and immune disorders. Irradiation of food as well as animals produces vitamin D and more than 90% of previtamin D3 synthesis in the skin occurs in the epidermis. Vitamin D receptor has been found in many cells including T and B lymphocytes, macrophages, mast cells, NK cells and Tregs, and it selectively binds with high affinity to its ligand. Vitamin D binds its receptor VDR, resulting in transcription of a number of genes playing a role in inhibition of MAPK. Its effect may be also mediated by the direct activation of PKC. Vitamin D has the ability to suppress inflammatory cytokines such as TNF, IL-1, IFN-gamma and IL-2; while it increases the generation of anti-inflammatory cytokines IL-4 and IL-10. In B cells, vitamin D3 have also been shown to suppress IgE antibody class switch partly through the inhibition of NF-kB. Here we discuss the relationship between vitamin D, immunity and skin disorders.

PROTEOMIC CHANGES OF RECOMBINANT YEAST: PHARMACO-INDUSTRIAL POTENTIAL

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The original yeast strain *Hansenula anomala* 2340 was implanted by low-energy nitrogen ion (N⁺) to obtain the mutant strain N6076. The mutant strain produced a red quinone compound, not synthesized by the parent strain. Two-dimensional fluorescence difference gel electrophoresis (2-D DIGE) and mass spectrometry (MS) were utilized to analyze the protein profile of the mutant strain N6076. The proteome changes were compared to those of the original strain to assess the amount of change that the metabolic pathways underwent in the mutant strain. The results indicated the detection of 57 different expressed proteins ($P < 0.05$) when the N6076 mutant strain was cultured in the liquid medium for 96 h as compared to that of the original strain. Of these different expressed protein spots, 27 were upregulated, and 30 were down-regulated. Also, 56 protein spots were identified with the aid of MALDI-TOF and tandem (TOF-TOF) MS. The protein score confidence interval (CI) of the protein profiling in the down-regulated protein spots 273 and 1294 were 81.371% and 12.864%, respectively, by bioinformatic analysis. This probably points to the fact that the irradiation by N⁺ contributed to the mutation of these two proteins.

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CORRELATION BETWEEN CLINICAL PATHOLOGY OF LUMINAL B BREAST CANCER AND DETERMINATION OF ESTROGEN RECEPTOR, PROGESTERONE RECEPTOR AND HER2 EXPRESSION COMBINED WITH NUCLEAR MORPHOLOGY

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D. Yin and Y Ma are joint first authors and contributed equally to this study

Breast cancer, one of the most common malignant tumors in females, draws little attention because of its untypical symptoms and signs, so the disease is usually confirmed too late, in an advanced stage. Based on the detection of nuclear morphology parameters of luminal B breast cancer, this study explored how pathological features relate to estrogen receptor (ER) and progesterone receptor (PR) expression of human epidermal growth factor receptor-2 (HER2). A quantity of 354 breast cancer specimens with follow-up records from the department of pathology in the First People's Hospital of Nantong and the Tumor Hospital of Nantong were selected as research subjects. Nuclear parameters of specimens stained by hematoxylin and eosin were measured by imaging analysis software. It was found that breast cancer can be divided into four types, luminal B, luminal A, HER2 over-expression and basal-like type based on immunohistochemical results of three antibodies, i.e, ER, PR and HER2. A total of 113 patients (31.8%) were confirmed with luminal B breast cancer, mostly in histological stage II; the difference of nuclear morphology was of statistical significance between ER+/PR+ and ER-/PR- ($P<0.05$), and most ER-/PR- was histologically confirmed as stage III, with lower survival rate than ER+/PR+ ($P<0.05$). Among these four subtypes of breast cancer, luminal B had the lowest brain metastasis rate, while HER2 over-expression subtype was found with the highest rate of lung and pleura metastasis. Besides, luminal B possessed longer disease-free survival (DFS) than basal-like ($P<0.05$) and longer total survival (OS) than HER2 over-expression ($P<0.05$) and basal-like subtypes ($P<0.05$). It can be concluded that detection of ER, PR and HER2 in combination with nuclear morphology is beneficial to evaluate treatment and prognosis of breast cancer.

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**EFFECT OF SEASONAL CHANGES ON TESTICULAR MORPHOLOGY AND
THE EXPRESSION OF CIRCADIAN CLOCK GENES IN JAPANESE WOOD MICE
(*APODEMUS SPECIOSUS*)**

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This study aimed to determine the seasonality of reproduction throughout the year in Japanese wood mice (*Apodemus speciosus*). The effect of seasonal changes on testicular morphology and the periodic expression of circadian clock genes in the hypothalamus and testes of male individuals was evaluated. We also examined the morphology of the testes and caudae epididymides of male mice. In addition, RT-PCR analysis was carried out with mRNA extracted from the hypothalamus and testes to evaluate the expression of the circadian clock genes *Clock*, *Bmal1*, *Per1*, and *Cry1*. The complete induction of testicular activity was detected from February to April and from August to October, with testes weight increasing with the completion of spermatogenesis (reproductive season). From May to early June and from November to early January, testicular weight declined, the seminiferous tubules reduced in size, spermatogenesis was arrested, and sperm were not produced (non-reproductive season). From mid-June to July and mid-January, the re-induction of testicular activity for spermatogenesis was observed in the seminiferous tubules (transitional season). Out of the four examined genes, *Cry1* had the highest expression level in both the hypothalamus and testes throughout the year, followed by *Bmal1*, *Per1*, and *Clock*. The expression of *Bmal1* was significantly lower in the hypothalamus and testes during the transitional season compared to the reproductive and non-reproductive seasons. *Cry1* transcript levels were also significantly lower in the hypothalamus and testes during the transitional season compared to the reproductive season. In conclusion, the results indicating changes in testicular morphology revealed annual reproductive, non-reproductive, and transmission periods in Japanese wood mice. When an increase in testicular activity was observed indicating the onset of the reproductive season, the mean day length was approximately 11–13 h. The expression of the circadian clock genes *Bmal1* and *Cry1* in the hypothalamus and testes during the reproductive season was significantly higher than that of the same genes during the transitional season. Consequently, completion of spermatogenesis occurred in the seminiferous tubules of Japanese wood mice testes during the reproductive period.

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HoxB7 PROMOTES GROWTH AND METASTASIS OF LUNG ADENOCARCINOMA CELLS THROUGH REGULATION OF THE TGF- β /SMAD3 SIGNALING

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HoxB7 is involved in cell migration and metastasis in many malignant tumors. But, the role of HoxB7 in lung adenocarcinoma has not been elucidated. In the present study, we aimed to clarify the function of HoxB7 in the progression of lung adenocarcinoma. The protein expression of HoxB7 was examined by immunohistochemical assay in human lung adenocarcinoma tissues, and lentivirus-mediated HoxB7 shRNA (Lv-shHoxB7) was transfected into lung adenocarcinoma cells to evaluate cell proliferation and invasive potential indicated by MTT and Transwell assays. As a result, the protein expression level of HoxB7 was increased in lung adenocarcinoma tissues compared with the adjacent non-tumor tissues (56.25% vs 31.25%, $P=0.014$), and was positively correlated with the lymph node metastasis in patients with lung adenocarcinoma ($P=0.036$). Moreover, knockdown of HoxB7 decreased the proliferation and invasion of lung adenocarcinoma cells followed by decreased expression of TGF- β /SMAD3, vascular endothelial growth factor A (VEGFA) and matrix metalloproteinase-2 (MMP-2). Taken together, our findings demonstrate that increased expression of HoxB7 is associated with tumor metastasis in patients with lung adenocarcinoma and HoxB7 may be implicated in promoting the development of lung adenocarcinoma through activation of the TGF- β /SMAD3 signaling.

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REPAIR EFFECTS OF UMBILICAL CORD MESENCHYMAL STEM CELLS ON PODOCYTE DAMAGE OF IgA NEPHROPATHY

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This study aimed to explore the influence of umbilical cord mesenchymal stem cells (UMSC) on stem cell homing and glomerular mesangial cell (GMC) after intravenous injection performed on mice tails with IgA nephropathy (IgAN) and its possible mechanism, which provide a new way and theoretical basis for the application of stem cell transplantation (SCT) in kidney disease treatment. Specific pathogen free (SPF) male Kunming mice were randomly divided into groups. A complex method applying bovine serum albumin (BSA) gavage, hypodermic injection of CCl_4 and lipopolysaccharide (LPS) was used for building IgAN mice model. In addition, vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF) and cluster of differentiation (CD) 44 were observed by Masson staining and detected with immunohistochemistry (IHC) to confirm homing and location of mesenchymal stem cells (MSCs). Moreover, Western Blot was used for detecting VEGF and CTGF so as to explore the possible mechanism of applying UMSC in treating IgAN. Masson staining indicated that fibrosis degree of MSCs in treatment group was significantly lower than in negative control group after stem cell treatment. Routine urine test explained that proteinuria in treatment group were (7.15 ± 0.31) , (4.87 ± 0.22) , (2.95 ± 0.16) g/24 h and (12.00 ± 1.38) g/24 h in model group ($P<0.05$). MSCs were observed to be located in glomerulus and renal interstitium by IHC detection of CD44 and IHC qualitative observation of VEGF and CTGF had different positive expressions in three groups. Furthermore, different expressions of VEGF and CTGF were observed quantitatively by Western Blot. Fibrosis degree of renal tissue relieves, hematuria and proteinuria eases and IgAN symptoms obviously improve after UMSC treatment, which hints that the treatment of HUMSC has protective effect on IgAN mice model.

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EFFECT OF INDOMETHACIN AND ITS COMPLEXES ON REPRODUCTIVE PERFORMANCE AND OXIDATIVE STRESS IN TESTIS AND STOMACH OF MALE ALBINO RATS WITH REFERENCE TO THEIR CHEMICAL CHARACTERIZATIONS

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Four new complexes of Hg (II), Pb (II), Sn (II) and Bi (III) with indomethacin drug ligand (IMC) were synthesized and characterized by using infrared, electronic, ¹H-NMR spectral, thermogravimetric and conductivity measurements. The IMC was found to act as bidentate chelating agent. IMC complexes coordinate through the oxygen of the carboxyl group. The molar ratio chelation is 1:2 (M²⁺:IMC) with general formula [M (IMC)₂], nH₂O for Hg (II), Pb(II) and Sn(II), but 1:3 for Bi(III) ions. Antibacterial screening of these heavy metal complexes against *Escherichia coli* (Gram-ve), *Bacillus subtilis* (Gram +ve) and anti-fungi (*Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus Flavus*) were investigated. In the present study, we found evidence suggesting that Bi³⁺/IMC possesses the capacity to protect the stomach, sperm, testes, cellular ATP, cellular NAD, INSL3, PGD2, PGE2 and antioxidant enzymes from deleterious actions of IMC.

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CAPACITIVE COUPLING ELECTRIC FIELDS IN THE TREATMENT OF VERTEBRAL COMPRESSION FRACTURES

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Positive effects of Capacitive Coupling Electric Field (CCEF) stimulation are described for several orthopedic indications such as the healing of recent fractures, non-unions and spinal fusion, due to the capacity to involve the up-regulation of osteopromotive factors. *In vitro* studies on MC3T3-E1 bone cells showed that CCEF acts opening the plasma membrane voltage gated calcium channels, thus increasing the cytosolic calcium concentration and the phospholipase A2 (PLA2) activity. Cytosolic calcium activates the calmodulin pathway, thus resulting in an up-regulated expression of osteogenic genes, such as transforming growth factor- β superfamily genes (TGF- β 1, - β 2 - β 3, bone morphogenetic protein-2 and -4), fibroblast growth factor (FGF)-2, osteocalcin (BGP) and alkaline phosphatase (ALP). PLA2 acts increasing the synthesis of Prostaglandin E2 (PGE2), which promotes osteogenesis by raising the cellular L-ascorbic acid uptake through the membrane carrier sodium vitamin C transporter-2 (SVCT-2). *In vivo*, Brighton et al. in a castration-induced osteoporosis animal model, demonstrated that CCEF was able to restore bone mass/unit volume in the rat vertebral body. To investigate the role of CCEF stimulation in vertebral bone marrow edema (VBME) its percentage was assessed in 24 patients with 25 acute vertebral compression fractures (VCFs) conservatively treated with CCEF (group A) or without CCEF (group B) using serial MR imaging follow-up at 0, 30, 60, 90 days. Pain and quality of life were assessed by visual analog scale (VAS) and Oswestry Low Back Disability Index (ODI) in the same periods. At 90 day follow-up the complete resolution of VBME was found only in group A ($p=0.0001$). A significant improvement of VAS ($p=0.007$) and ODI ($p=0.002$) was also observed in group A. This preliminary observational study shows that patients treated with CCEF stimulation present an improvement of clinical symptoms with faster fracture healing and a complete VBME resolution.

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HYALURONIC ACID: THE USE OF ITS PRECURSOR IN SKIN BIO-STIMULATION

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Bio-stimulation is an injective therapy aimed to boost the anabolic functions of dermal fibroblasts to obtain skin improvement. It can be achieved with multiple intradermal injections (0.05–0.1 ml each) of a solution of 400 mg (3 ml) of injectable glucosamine sulphate, plus 5.623 mg (3 ml) of polideoxyribonucleotide, 1 ml of lidocaine and 0.5–1 ml of sodium bicarbonate, to repeat every 7, 14, 21, and 28 days. The administration of glucosamine sulphate to skin fibroblasts is believed to lead to its incorporation in glycosaminoglycans, and thereby to the stimulation of extracellular matrix synthesis, whereas polideoxyribonucleotide possesses anti-inflammatory and regenerative capability. This study aims to elucidate the *in-vitro* effects of this treatment by studying what happens to several genes related to connective tissue integrity. Human dermal fibroblasts were seeded in a culture medium enriched with either two drugs alone or combined: glucosamine sulphate and/or polideoxyribonucleotide. After the end of the exposure time of 24 h, 48 h, and 72 h, the cells were trypsinized and lysed for RNA extraction. Reverse transcription to cDNA was performed directly from cultured cell lysate. Finally, the cDNA was amplified by real-time PCR and a panel of genes involved in dermal integrity was tested. Gene expression of Hyaluronan synthase 1 (HAS1), Elastine (ELN), Insulin like growth factor 1 (IGF1), Growth differentiation factor 6 (GDF6) and of a series of catabolic enzymes, such as Metalloproteases (MMP) 2, 3 and 13, the neutrophyl expressed Elastase (ELANE) and the Hyaluronidase 1 (HYAL1) were tested after 24, 48 and 72 hours of exposure to glucosamine sulphate and polideoxyribonucleotide alone or combined. All the tested genes but one were up-regulated. A negative synergism on several enzymes (particularly appreciable for Insulin-like growth factor 1 and metalloprotease 13) was observed when the two drugs were delivered together. Glucosamine sulphate acts not only as building block in the biosynthesis of glycosaminoglycan chains, but also as a booster of hyaluronan synthase 1. The association of glucosamine sulphate and polideoxyribonucleotide, used in bio-stimulation therapy protocol, has a negative synergism on catabolic genes in dermal fibroblast cultures. The present observations produce further insight into the effects of glucosamine sulphate in the biosynthesis of glycosaminoglycan chains.

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PAPILLARY THYROID CANCER IS CHARACTERIZED BY ALTERED EXPRESSION OF GENES INVOLVED IN THE SUMOYLATION PROCESS

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Small Ubiquitin-like MOdifier (SUMO) proteins are small protein modifiers capable of regulating cellular localization and function of target proteins. Over the last few years, a relevant role has been demonstrated for sumoylation in the modulation of important cellular processes, including gene transcription, DNA repair, cell-cycle regulation and apoptosis. Components of the sumoylation machinery have been found deregulated in different human cancers, and are thought to significantly affect cancer cell progression. In the present study we sought to analyze the expression of all the components of the sumoylation machinery in a case study comprising 77 papillary thyroid cancers (PTC) and normal matched tissues. In particular, we evaluated the expression of the SENP1 to SENP8 (SENtrin-specific proteases), SAE1 (SUMO1 activating enzyme subunit 1), UBA2 (UBiquitin-like modifier activating enzyme 2), UBC9 (UBiquitin conjugating enzyme 9), RanBP2 (RAN binding protein 2), MSMCE2 (Non-SMC element 2), CBX4 (ChromoBoX homolog 4), PIAS1 to PIAS4 (protein inhibitor of activated STAT), ZMIZ1 (zinc finger, MIZ-type containing 1) and ZMIZ2 (Zinc finger, MIZ-type containing 2) by means of quantitative RT-PCR. In most of the PTC examined we observed a significant alteration in the mRNAs of SENP8, ZMIZ1, SAE1, PIAS1 and PIAS2. These tended to be reduced in about 50 to 66% of cases, and unchanged or increased in the remaining ones. Univariate and Kaplan-Mayer analyses documented the lack of association between the expression of the above 5 genes and clinicopathological parameters. Only SAE1 was significantly higher in female PTC tissues, in respect to male PTC tissues ($p=0.021$), and SENP8 was significantly lower in TNM stages III-V, with respect to stages I-II ($p=0.047$). In conclusion, we demonstrated that the expression of SENP8, SAE1, PIAS1, PIAS2 and ZMIZ1 is deregulated in the majority of PTC tissues, likely contributing to the PTC phenotype. However, differently from other human cancers, their mRNA level does not represent a prognostic biomarker in PTC patients.

CD133 EXPRESSION COULD BE A PREDICTIVE MARKER OF PERIODONTAL REGENERATION

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Periodontal regeneration needs formation of new connective tissue at the root surface, involving periodontal fibre development and angiogenesis. CD133 or prominin-1, is an important regulator of apoptosis, proliferation and angiogenesis. CD133 positive cells seem to be influenced in number and distribution by periodontal inflammatory changes. Studies showed different clinical and radiographic outcomes achieved with the used of Demineralized Freeze-Dried Bone Allografts (DFDBA) for periodontal intrabony defects treatment. Our aim was to investigate the relationship between CD133 expression in gingival biopsies before periodontal treatment and periodontal tissue response in the same site at 12 months post-surgery. We selected fifty-six patients with at least one intrabony defect with clinical attachment level (CAL) \geq 6 mm and needing periodontal regeneration. A gingival biopsy for each patient was obtained for CD133 immunostaining. Clinical and radiographical parameters were taken at baseline and 12 months post-surgery. We found a positive correlation between gingival CD133 expression and CAL gain achieved by use of DFDBA and measured 12 months post-surgery. Our results suggest that gingival CD133 expression could be a predictive marker of favourable periodontal healing. The CAL gain after periodontal regeneration seems to be related with a native gingival regenerative capacity.

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LETTER TO THE EDITOR

INHIBITORY ACTION OF CoCl₂-INDUCED MCF-7 CELL HYPOXIA MODEL OF BREAST CANCER AND ITS INFLUENCE ON VASCULAR ENDOTHELIAL GROWTH FACTORM. ZHANG¹, R. MA² and Q. LI¹

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Breast cancer, a malignant tumor frequently occurring in females, is traditionally treated with excision. In the search for a new treatment, we analyzed the influence of CoCl₂ on MCF-7 cell proliferation of breast cancer and tumor angiogenesis factor and discussed the results. Having applied CoCl₂ as chemical hypoxia-induced agent, *in-vitro* MCF-7 cell hypoxia model of breast cancer was established, after which methyl thiazolyl tetrazolium (MTT) staining was performed in detecting inhibitory action of CoCl₂ to proliferation of MCF-7 cells cultured *in-vitro*, and inverted phase contrast microscope was adopted to observe morphological changes of MCF-7 cell in hypoxia model. Furthermore, reverse transcription-polymerase chain reaction (RT-PCR) was made to determine how CoCl₂ influences mRNA expression changes of hypoxia inducible factor-1 α (HIF-1 α), chemokine receptor-4 (CXCR4) and vascular endothelial growth factor (VEGF) in MCF-7 cells. Western blot was designed to study and record data on the influence of CoCl₂ on protein expression changes of HIF-1 α , CXCR4 and VEGF. As a result, CoCl₂ was proved to control MCF-7 cell proliferation and increase expression of HIF-1 α , CXCR4 and VEGF.

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LETTER TO THE EDITOR

TRANSPLANTATION OF HYPERTHERMIC PRECONDITIONING OLFACTORY ENSHEATHING CELLS COMBINED WITH NEURAL STEM CELLS IN THE TREATMENT OF CENTRAL NERVE INJURY

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This study aims to observe the effect of the transplantation of hyperthermic preconditioning (HPC) olfactory ensheathing cells (OECs) at 40°C combined with neural stem cells (NSCs) in the treatment of spinal cord injury (SCI), based on the OECs and NSCs taken from the olfactory bulbs and cerebral cortex of newborn rats. Forty-two female Sprague Dawley (SD) rats were randomly divided into: control group, NSCs+OECs without HPC group and NSCs+HPC OECs group. Firstly, hemisected spinal cord injury model was established; the motor function recovery of the right lower limb of the rats was compared by Basso-Beattie-Bresnahan rating (BBB rating), climbing score and running time on a rotating platform during the whole experiment. At one day, two weeks and four weeks after transplantation, two rats were randomly selected from each group for section preparation. Hematoxylin and eosin (HE) staining was performed on the sections to observe and analyze the pathological changes of the spinal cord tissue, and bromodeoxyuridine (BrdU) labeling was used to observe the distribution of transplanted cells. The results demonstrated that, BBB score of the rats that were treated by transplantation of NSCs combined with HPC OECs was distinctly improved; a rapid increase of BBB score was found two weeks after transplantation, while BBB score had slightly increased six weeks later. BBB score of the control group and the NSCs+OECs without HPC group was found with a slight increase, especially in the control group. BBB score of NSCs+HPC OECs was significantly higher than in the control group and the NSCs+OECs without HPC group at the 2nd, 4th, 6th, 8th and 12th week after treatment ($P<0.05$). Climbing tests and detection of running time after 4 weeks and 6 weeks demonstrated that, the recovery of limb function of the NSCs+HPC OECs group was better than the other groups ($P<0.05$). HE staining results of NSCs+HPC OECs indicated that, cells of the spinal cord were neatly arranged, close to normal. BrdU labeling results revealed that, transplanted cells were found in injury tissue, indicating that they were involved in the spinal cord repair. This study proves that, the effect of NSCs combined with HPC OECs in the treatment of SCI is better than NSCs combined with OECs without HPC, and the ratio of NSCs differentiating to neuron after inducing HPC OECs supernate is higher than that after inducing OECs supernate without HPC.

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*LETTER TO THE EDITOR***CLOSED FEMORAL NAILING WITH THE TECHNIQUE OF USING A NEW FEMORAL
DISTRACTOR: A PRELIMINARY REPORT**

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This study introduces the application of a new femoral distractor in the treatment of femoral fracture restoration with internal fixation of intramedullary nail. Sixty-three patients with femoral fracture from the Affiliated Hospital of Binzhou Medical University underwent femoral fracture restoration with the new femoral distractor in combination with internal fixation of an intramedullary nail from June 2011 to March 2014. There were 18 cases of proximal femur fractures, 44 cases of middle femoral shaft fractures and 1 case of distal femur fracture. Follow-up was on the 4th, 6th, 8th, 12th, 16th and 24th week after operation. All 63 patients successfully underwent the surgery and the steel needles used did not cause injury to the adjacent vessels or nerves. Five cases had to have steel needles reinserted, as they had failed in the distraction reduction due to being unsteadily fixed because of an improper position. Patients were followed up for 10~24 months (mean 16 months), and the total healing rate was 100%. Operative time was 93.5 minutes averagely. Average time of patients' exposure to X-ray was 26.8 seconds. Bleeding volume was averagely 219.1 ml. There were no complications either during the operations or after them. All cases healed within 12 weeks (average 7.6 weeks). This study proves that, the new femoral distractor can help the closed reduction of fractures in treating femoral fractures with intramedullary nails to avoid the inconvenience of applying traction tables and the occurrence of potential complications.

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LETTER TO THE EDITOR

NEUROENDOCRINE FUNCTIONS OF PUERPERAE WITH POSTPARTUM DEPRESSION AGGRAVATED BY STRESSFUL CHILDBIRTH-RELATED EVENTS

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In the period of gestation, delivery and post-delivery, fear and tension produced in puerperae are likely to evolve into depression as they worry too much about delivery pain. In recent years, it has been noted that stressful events during this period aggravate postpartum depression. To discuss the effect of these childbirth-related stressful events on neuroendocrine functions of patients with postpartum depression, 300 full-term puerperae who had been admitted to the Beijing Obstetrics and Gynecology Hospital, Capital Medical University between October, 2011 and October, 2013 and who had suffered from stressful childbirth-related events were enrolled as a study group. This group was divided into six subgroups, i.e., A, B, C, D, E and F, based on the number of stressful events they had suffered which were labeled by numbers 1 to 6. Additionally, 100 puerperae from the same hospital who had not suffered from childbirth-related stressful events were taken as controls. Relevant clinical indexes, including serum adrenocorticotrophic hormone (ACTH), plasma 5-hydroxytryptamine (5-HT), noradrenaline ELISA (NE), dopamine (DA) and cortisol level were measured and compared. It was found that incidence probability of postpartum depression was significantly different between the study group (13.67%, 41/300) and the control group (7%, 7/100). Moreover, the incidence probability of postpartum depression of puerperae suffering from no less than 4 childbirth-related stressful events was higher than those suffering from no more than 3, and the difference was statistically significant ($P < 0.05$). Thus, stress disorders caused by these events are one of the important pathogenic factors of postpartum depression.

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LETTER TO THE EDITOR

IMP-3 EXPRESSION IN BENIGN MELANOCYTIC NEVI, DYSPLASTIC NEVI AND MALIGNANT MELANOMA: PRELIMINARY FINDINGS IN BULGARIAN PATIENTS

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IMP-3 is generally considered as an oncofetal protein, which plays a critical role in regulation of cell proliferation via an IGF-II-dependent pathway in K562 leukemia cells. IMP-3 expression has been detected in malignancies with various origins, while its appearance in adult tissue is generally considered abnormal, with some exceptions. IMP3 is also considered a prognostic biomarker in patients with renal cell carcinoma and clear-cell type ovarian carcinoma, hepatocellular carcinoma, pancreatic ductal adenocarcinoma and in patients with poorly differentiated thyroid carcinoma and uterine cervical carcinomas, testicular cancer and malignant melanoma. To our knowledge, no more than 4 PubMed-indexed studies have investigated the expression of IMP-3 in melanocytic lesions, namely its role in the differentiation between benign and malignant neoplasms. We investigated the expression of IMP-3 in a small series of benign melanocytic lesions, dysplastic nevi and melanomas, aiming to establish its significance as a marker for their distinction, comparing the results with those from the literature. IMP-3 immunostaining was performed in 30 melanocytic lesions: 10 malignant melanomas, 10 dysplastic nevi and 10 benign melanocytic nevi. Our results revealed expression in 20% of dysplastic lesions and 40% of melanoma cases, while none of the benign nevi showed positive expression. These data contradict some of the results from other studies and raise some questions regarding the correlation between IMP-3 and the degree of dysplasia of melanocytic nevi, as well as its potential relationship with prognostic parameters in melanoma, including tumor thickness and mitotic rate. Our results suggest that IMP-3 expression could be only an auxiliary marker for differentiation between dysplastic nevi and benign nevi, since although it is not expressed in all dysplastic lesions, staining correlates with the degree of dysplasia/atypia. It seems that IMP-3 expression is not a useful discriminator between dysplastic nevi and melanoma nor a good prognostic marker in melanoma.

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LETTER TO THE EDITOR

TOPICAL CORTICOSTEROIDS BUT NOT CALCINEURIN INHIBITORS INDUCED ATROPHY AFTER FOUR WEEKS

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Reflectance confocal microscopy (RCM) is a non-invasive, *in vivo* technique for real-time imaging of the epidermis and superficial dermis at the cellular resolution. We performed a pilot study focusing on the evaluation of the effect of topical corticosteroids and calcineurin inhibitors on the epidermis of patients with atopic dermatitis (AD). The effect was assessed by RCM. A total of 45 patients with AD took part in the study. Patients were selected according to the standardized protocol and divided into two groups. Twenty-three patients used methylprednisolone aceponat topically on the skin with lesions of AD once a day for three months (group A). Twenty-one patients applied topical tacrolimus on the skin with lesions of AD twice a day for three months (B). RCM imaging was performed on the day of initiating the study (T0), then after one (T1), two (T2) and three months (T3). In group A, there was a visible decrease of the stratum corneum and the epidermis thickness which was statistically significant. In comparison, in group B, such changes were not noted and the differences between the groups in time course were statistically significant. In group A, an increase in the percentage of blurred keratinocytes in the stratum spinosum was also recorded, especially between the first (T0) and the second visit (T1). RCM is a useful method for evaluating the changes in epidermis due to the different topical treatment in patients with AD.

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*LETTER TO THE EDITOR***RECTAL IMPACTION DUE TO PRICKLY PEAR SEEDS BEZOAR:
A CASE REPORT**

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Fecal impaction is the third cause of lower gastrointestinal tract obstruction after strictures for colon cancer and postoperative adhesions. A rapid diagnosis is necessary to avoid complications due to intestinal obstruction. Rectal phytobezoar due to prickly pear fruit seeds are an extremely rare entity, in the literature about twenty similar cases are described. Prickly pears are common in many countries, even in the Mediterranean area. When the ingestion of their fruit is excessive, this can be harmful, leading to the formation of phytobezoar causing fecal impaction. We describe the first case of phytobezoar due to prickly pear fruit seeds in continental Europe: a 76-year-old Italian female who ingested almost 40 prickly pear fruit leading to the composition of a large rectal phytobezoar. The patient presented clinically with fecal impaction, diagnosed by imaging and successfully treated by rectal irrigation and manual disimpaction. Our aim is to remind the physicians of these risks in evaluating patients with intestinal obstruction, when there is positive anamnesis for provenience from some areas in which these fruits are eaten. We also want to underline the role of Imaging Multi Detector Computed Tomography (MDCT) in the diagnosis of these very uncommon entities.

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LETTER TO THE EDITOR

DENTAL PULP STEM CELLS AND HUMAN PERIAPICAL CYST MESENCHYMAL STEM CELLS IN BONE TISSUE REGENERATION: COMPARISON OF BASAL AND OSTEOGENIC DIFFERENTIATED GENE EXPRESSION OF A NEWLY DISCOVERED MESENCHYMAL STEM CELL LINEAGE

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Bone regeneration is an interesting field of biomedicine. The most recent studies are aimed to achieve a bone regeneration using mesenchymal stem cells (MSCs) taken from more accessible sites: oral and dental tissues have been widely investigated as a rich accessible source of MSCs. Dental Pulp Stem Cells (DPSCs) and human Periapical Cysts Mesenchymal Stem Cells (hPCy-MSCs) represent the new generation MSCs. The aim of this study is to compare the gene expression of these two innovative cell types to highlight the advantages of their use in bone regeneration. The harvesting, culturing and differentiating of cells isolated from dental pulp as well as from periapical cystic tissue were carried out as described in previously published reports. qRT-PCR analyses were performed on osteogenic genes in undifferentiated and osteogenic differentiated cells of DPSC and hPCy-MSC lineage. Real-time RT-PCR data suggested that both DPSCs and hPCy-MSCs cultured in osteogenic media are able to differentiate into osteoblast/odontoblast-like cells: however, some differences indicated that DPSCs seem to be directed more towards dentinogenesis, while hPCy-MSCs seem to be directed more towards osteogenesis.

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LETTER TO THE EDITOR

CLODRONATE: OLD DRUG, NEW USES

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Clodronate (CLO) is a bisphosphonate (BP) with proved efficacy in the treatment of osteoporosis. The reason of its activity is the anti-resorptive action, which is a common characteristic of BPs. Contrary to other BPs, CLO has a relatively low affinity for bone and a peculiar mechanism of action. CLO is effective in several diseases associated to excessive bone resorption as bone Paget's disease and CRPS type I. Moreover, there are data showing activity of CLO in the erosive osteoarthritis of the hands, in the osteoarthritis of the knees, in the treatment of extra-articular calcifications and in preventing the mobilization of knee and hip prosthesis.

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LETTER TO THE EDITOR

TELOMERE AND TELOMERASE MODULATION BY BERGAMOT POLYPHENOLIC FRACTION IN EXPERIMENTAL PHOTOAGEING IN HUMAN KERATINOCYTES

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Photoageing represents the addition of extrinsic chronic ultraviolet radiation-induced damage on intrinsic ageing and accounts for most age-associated changes in skin appearance. In this study, we evaluated the effect of 38% BPF, a highly concentrated extract of the bergamot fruit (*Citrus bergamia*) on UVB-induced photoageing by examining inflammatory cytokine expression, telomere length/telomerase alterations and cellular viability in human immortalized HaCaT keratinocytes. Our results suggest that 38% BPF protects HaCaT cells against UVB-induced oxidative stress and markers of photoageing in a dose-dependent manner and could be a useful supplement in skin care products. Together with antioxidant properties, BPF, a highly concentrated extract of the bergamot fruit, appears to modulate basic cellular signal transduction pathways leading to anti-proliferative, anti-aging and immune modulating responses.

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LETTER TO THE EDITOR

NORMAL NUTRITIONAL COMPONENTS AND EFFECTS ON BONE METABOLISM IN PREVENTION OF OSTEOPOROSIS

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Osteoporosis is the most common bone disease, affecting millions of people and causing a high risk of fractures and a loss of quality of life. It is characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. A primary method of prevention, in order to reduce the risk of fractures, is represented by an appropriate lifestyle and a correct diet. There are potentially numerous nutrients and dietary components that can influence bone health, and these range from macronutrients to micronutrients as well as bioactive food ingredients. The purpose of this review is to overview osteoporosis, including its definition, etiology, and incidence, and then provide some information on possible dietary strategies for optimizing bone health and preventing osteoporosis. A correct diet to prevent osteoporosis should contain adequate amounts of calcium, vitamins D and K, protein, and fatty acids. The effects of these elements are briefly discussed, reporting on their correlation with bone benefits.

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LETTER TO THE EDITOR

**METABOLIC BONE CHANGES IN OSTEOARTHRITIS:
THE ROLE OF IMAGING AND PATHOGENETIC INTERPRETATION**E. SILVESTRI¹, A. CORAZZA², L. MOLFETTA³ and G. GARLASCHI²

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Osteoarthritis (OA) is the most prevalent chronic joint disease and one of the major causes of disability in the adult population. Although OA is considered a progressive degenerative process which involves the whole joint, articular cartilage and subchondral bone play a determinant role in its pathogenesis. In particular, metabolic-triggered subchondral bone damage, together with biochemical markers, are referred as important indicators of the disease. Magnetic resonance (MR) is the best imaging technique to detect and characterize such bone abnormalities. It represents an effective method through which to not only diagnose, describe and follow the course of OA but also to deepen our understanding of the natural history of the disease, with the ultimate purpose of attaining improved outcome in terms of therapy and prognosis. Even though MR has enormous potential, some diagnostic pitfalls may occur in clinical practice, hence an accurate clinical assessment of the patient is mandatory in combination with optimal imaging evaluation.

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