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International Conference on
Stem Cells and Regenerative Medicine
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2nd World Congress on
Pediatrics and Child care

November 06-07, 2019 | Tokyo, Japan

Poster

30 YEARS
OF EXCELLENCE
IN MEDICAL
PUBLISHING

Electrophoretic deposition and characteristics of chitosan/AgNPs coatings on the Ti13Zr13Nb alloy

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Introduction: The surface of materials intended for implants is modified, among others to improve their biocompatibility and providing protection against bacteria. It is important to provide long-term protection against bacterial colonization, hence intensive work on the production of coatings that will release the drug substance in a controlled manner. Chitosan belongs to the group of so-called "intelligent" biopolymers, because it reacts to pH changes. Chitosan coatings can therefore be a matrix that will release the drug substance when the inflammation occurs, which is associated with a decrease in the pH value of the environment of peri-implant tissues.

Methodology: As part of this work, chitosan coatings with silver nanoparticles were produced by electrophoretic method on the surface of the Ti-13Zr-13Nb titanium alloy, previously anodized electrochemically to obtain a nanotube oxide layer. Microstructure, morphology and phase composition were examined with scanning electron microscopy and X-ray diffractometer. The mechanical behavior was studied by nanoindentation and nanoscratch tests. The silver dissolution rate in simulated body fluid was measured with atomic absorption spectrometry.

Results: The produced nanotubes were highly ordered. The average diameter of nanotubes was about 40 nm, while their length - about 1 μm. The obtained composite coatings were characterized by high homogeneity, silver nanoparticles were quite evenly distributed in the coating, however, they tended to form agglomerates. Mechanical tests showed that the coatings are characterized by relatively high values of parameters such as: hardness and Young's modulus, (1.24 ± 0.16) GPa and (52.29 ± 3.73) GPa respectively. The adhesion of the coatings to the substrate was also satisfactory. The mean value of the critical force resulting in the total removal of the composite coating from the substrate was (155.03 ± 29.89) mN. The concentration of released silver reached a level sufficient to combat bacteria.

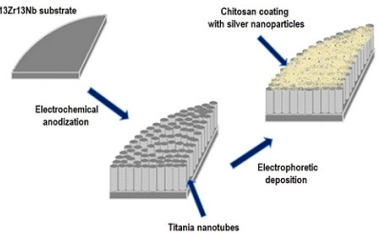

Figure 1. Graphical abstract

Biography

Lukasz Pawlowski is PhD student at Gdansk University of Technology, Department of Materials Engineering and Bonding, Biomaterials Group, Poland. His doctoral thesis concerns surface modification of titanium alloys mainly by electrophoretic deposition of composite coatings, which release drug substance in a controlled manner.

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e-Poster

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Stem cell growth and differentiation factors from zebrafish embryo and their role as epigenetic regulators in hair regeneration: Results after their transdermal administration by cryopass laser treatment

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Introduction: Previous studies, conducted over many years in our laboratories on zebrafish embryos, allowed the identification of precise moments of stem cell differentiation in which a lot of genes switch on and off, a sign that the genome is undergoing substantial changes in gene expression. The factors of the early developmental stage of zebrafish embryo were able to regulate the stem cell expression of multipotency, enhancing the stemness genes Oct-4, Sox-2 and c-Myc. In addition to affecting stemness genes which maintain stem cell identity, these factors taken in a primarily multiplicative stage also elicited transcriptional activation of two major mechanisms capable of opposing stem cell senescence, including the gene expression of TERT, the catalytic subunit of telomerase and the transcription of Bmi1, a Trithorax family of repressors which act as essential factors for self-renewal of adult stem cells and as key telomerase-independent repressors of cell aging.

On the contrary, the molecules taken during differentiation events are able to reprogramming pathological stem cells. On the basis of the researches about stem cell rejuvenation ad differentiation many studies were made. In the present study we present the clinical results on twenty men aged between 46 and 67 (average age 57) with androgenetic alopecia. They were treated with stem cell growth and differentiation factors from zebrafish embryo using cryopass-laser treatment for the transdermal administration. The materials and methods to prepare the zebrafish extracts and about the use of cryopass laser were already described.

Results: All the patients demonstrated an initial regeneration of hair in the form of a soft fleece after the first treatment. This regeneration was consolidated with subsequent treatments and after about 10 treatments the hair took on a consistency of adult and pigmented hair. The treatment did not have any adverse effect and was very well accepted by patients who were satisfied with the obtained results.

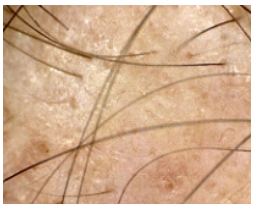

Figure 1. A patient before the treatment with stem cell growth and differentiation factors

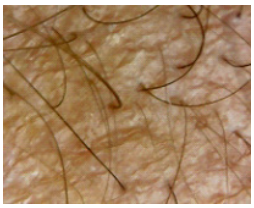

Figure 2. The same patient after the treatment

Biography

Biava PM is author of many scientific publication and 8 books about reprogramming pathological and normal stem cell.

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Accepted Abstracts

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Identification of a population of quiescent pluripotent stem cells within peripheral nerves

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Evidence from our laboratory has documented a large population of quiescent stem cells within peripheral nerves. In response to nerve injury, or stimulation with the cytokines (eg BMP2), these cells proliferate and generate populations of pluripotent stem cells, expressing Sox2, Klf4, Oct4 and c-Myc (verified by double stain immunohistochemistry and by real time PCR). These 4 markers are the transcription factors that confer embryonic pluripotency (Cell 126: 663, 2006). We call them nerve derived pluripotent stem cells, or NEDAPS cells. The cells propagate well in restrictive media and are readily induced to form tissues from all 3 germ layers. We hypothesize that pluripotent stem cells are indeed residing in peripheral nerves and they represent the central feature in an important and previously unknown universal pathway for tissue repair. Nerves are nearly ubiquitous in the body, from the cornea of the eye to every hair follicle. Thus, we believe that nerve injury and the consequent proliferation of these stem cells, occurs following essentially any injury. The cell of origin for these pluripotent stem cells are the Schwann cells, which have long been known to have remarkable plasticity, demonstrated by their behavior after a nerve transection. We believe that we have uncovered a previously unknown universal pathway for healing.

We will show data documenting the induction and successful culture of these unique new pluripotent cells from three mammalian species, mouse, rat and human and demonstrate their directed differentiation into osteoblasts, endothelial cells, primitive nerve cells, definitive endoderm, brown fat and fibroblasts as demonstrated by morphology, immunohistochemical staining and by real time polymerase chain reaction (RT-PCR) data to document cell specific gene expression.

Stem cell biology is a field that has recently seen an explosion of new work. stimulated by Dr Yamanaka's remarkable discovery that induced pluripotent stem cells (iPCs), or cells capable of differentiating into any cell type, could be created from fully differentiated cells by forcing expression of the genes for only 4 transcription factors (listed above), most often by the use of retrovirus vectors (Cell 126: 663,2006). Such iPCs are being widely studied as possible sources of cells for the treatment of human disease. This work has been hampered by issues of malignant transformation of iPCs and by immune rejection of these "non-self" cells. Previous claims to successful identification of cells with universal differentiation from non-gonadal adult tissue have sadly resulted in some notable and well publicized scandals, involving fabricated data). These scandals have understandably created a skeptical audience for us. Such pluripotent stem cells are thought not to exist in adult animals (SciON 311: 814 2006) and until the recent discovery of these cells by our group, we believed the same.

We propose that this new knowledge will also explain the puzzling and vexing clinical problem of impaired wound healing experienced by severely diabetic patients and victims of leprosy. We suggest that in the severe depletion or absence of Schwann cells due to the severe neuropathies caused by these illnesses, essentially makes wound healing impossible. The other implication of this discovery is that we may now have a straightforward opportunity to obtain individual specific "self-to-self" stem cell treatments based on a minimally invasive biopsy of a nonessential peripheral neve of a specific patient in need, from which NEDAPS cells could be easily propagated *ex vivo*. These NEDAPS cells could be differentiated into cells specifically needed by the individual patient who provided the nerve tissue. We suspect that this scheme will bypass the risk of malignant transformation, as well as immune rejection. This method has been successfully applied to a skin healing model, as well as fracture healing models.

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Small mobile stem cells produce strong angiogenic factors leading to tissue remodeling into macro vessels

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Current *in vitro* angiogenesis assays capture only early stages of micro- vessel formation. Human derived small mobile stem (SMS) cells produce matricellular proteins that induce human endothelial cells to form micro and macro vessels. SMS cell derived extracellular matrix is able, in the absence of any other cell type, to effect human primary endothelial cells microscopic differentiation and macroscopic tissue organization. This allows the *in vitro* capturing and monitoring of complex multi-stage, multi-step processes of developing micro vessels into macro-vessels. Notably, the cell differentiation and tissue organization create stable large vessels that become visible to the naked eye. This capability would provide, first-time, ample opportunities for interrogating pro or anti- angiogenesis factors, *in vitro*, at significantly more mature stages of vessel formation.

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Immunity boost and increased lifespan of tumor necrosis factor-α/CD40 ligand-engineered mesenchymal stem cells administred in mice

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The interaction between mesenchymal stem cells (MSCs) and dendritic cells (DCs) affects T cell development and function. Further, the chemotactic capacity of MSCs, their interaction with the tumor microenvironment and the intervention of immune-stimulatory molecules suggest possible exploitation of tumor necrosis factor-α (TNF-α) and CD40 ligand (CD40L) to genetically modify MSCs for enhanced cancer therapy. Both DCs and MSCs were isolated from BALB/c mice. DCs were then cocultured with MSCs transduced with TNF-α and/or CD40L [(TNF-α/CD40L)-MSCs]. Major DCs' maturation markers, DC and T cell cytokines such as interleukin-4, -6, -10, -12, TNF-α, tumor growth factor-β, as well as T cell proliferation, were assessed. Meantime, a BALB/c mouse breast tumor model was inducted by injecting 4T1 cells subcutaneously. Mice (n = 10) in each well-defined test groups (n = 13) were cotreated with DCs and/or (TNF-α/CD40L)-MSCs. The controls included untreated, empty vector-MSC, DC-lipopolysaccharide and immature DC mouse groups. Eventually, cytokine levels from murine splenocytes, as well as tumor volume and survival of mice, were assessed. Compared with the corresponding controls, both *in vitro* and *in vivo* analyses showed induction of T helper 1 (Th1) as well as suppression of Th2 and Treg responses in test groups, which led to a valuable antitumor immune response. Further, the longest mouse survival was observed in mouse groups that were administered with DCs plus (TNF-α/CD40L)-MSCs. In our experimental setting, the present pioneered study demonstrates that concomitant genetic modification of MSCs with TNF-α and CD40L optimized the antitumor immunity response in the presence of DCs, meantime increasing the mouse lifespan.

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Stem cells for treatment of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder leading to the death of motoneurons (MN). In our preclinical study human mesenchymal stem cells (hMSCs), were delivered intrathecally into SOD1 G93A transgenic rats. Survival in the hMSC-treated group was prolonged and rats showed better motility and grip strength. We found greater numbers of perineuronal nets (PNNs) in the hMSCs-treated animals and MSCs have antiapoptotic and immunomodulatory effects. The clinical study was designed as a prospective, non-randomized, open-label study (phase I/IIa, EudraCT No. 2011-000362-35) to assess the safety and efficacy of autologous BM-MSC in the treatment of ALS. BM-MSC were applied via lumbar puncture into the cerebrospinal fluid. During the 18-month follow-up period no serious adverse reactions or new cerebrospinal pathology on MR examinations were observed. The clinical outcome was evaluated by an ALS functional rating scale (ALSFRS), norris spinal and bulbar scale (NSS and NSB), forced vital capacity (FVC) and weakness scale (WS). In almost 80% of patients FVC values remained above 60% for a time period of 12 months. A group of 14 patients, with remarkable pretreatment decline in functional scales (ALSFRS + NSS), had significant reduction/stabilization in their total functional score decline at 3 months after application ($p<0.02$) that persisted for 6 months ($p<0.05$). In about 80% of the patients, FVC values remained stable or above 70% for a time period of 9 months. These results demonstrate that the intrathecal application of BM-MSC in ALS patients is a safe procedure and that it can slow down progression of the disease.

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Liver tissues regenerated from human tooth treats liver failure of rat cirrhosis model and swine NASH model

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Cadaveric or live-donor liver transplant is only the treatment for severe liver condition. However, the number of transplantations is very limited because of fewer available organs than number of the patients on the waiting list. The liver regeneration might be one of the alternative. Several clinical studies employed mesenchymal stem cells from blood, adipose tissue or others to transplant without differentiating the cells. However, Transplantation of these cells can only slow decline of hepatic function, but they cannot treat the conditions of the liver. The objective of adult stem cell transplantations might be to launch "bridge to transplant" strategy rather than treating liver condition. We have shown that human dental pulp stem cell demonstrates huge potential to treat lethal liver conditions. We have previously reported we treated the biliary liver cirrhosis and acute liver injury in nude rats with transplanting the regenerated liver tissues originated from human dental pulp. One of the most prevailing liver condition is non-alcoholic steatohepatitis (NASH). Hence the objectives of the research is to evaluate the clinical possibility of our transplantation protocols using swine model of progressive liver failure developed from NASH. After four weeks of transplantation of hepatocytes described from human tooth into the spleen of 6 swine with the failure under immune suppression, we found the secondary liver in the spleen was produced, as well the regenerated liver was produced using the original liver as scaffold. Biliary ducts are reproduced with human tissues only 4 weeks after the transplantation. Serum albumin level recovered from 1.5g/dL to over 3.0g/dL. HPT, choline esterase, collagen type IV, ALT and others have been dramatically improved. But any of the positive control has shown no change.

Following above we also treated rat cirrhosis model.

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Gradient photothermal field for precisely directing cell sheet detachment

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Traditional cell-based medical therapeutics are usually performed with the enzymes (trypsin) to transfer cells from culture dishes to the wounds, which destroy the extracellular matrix (ECM) and reduce cell efficacy, cell activity and cell integrity. Technologies for transferring cell sheets with a completely integrated ECM and intact cell–cell junctions have been established for applications in surgical operations and regenerative medicine, such as ocular regeneration, cardiac tissue regeneration and bladder augmentation. Developments of cell sheet engineering are in progress aimed at commercial applications. However, there are still a lot of problems in terms of practical operation. For example, the resultant cell sheets are easily crumpled, wrinkled and tangled due to the uncontrollable detachment and disturbance from hydrodynamic forces. For more delicate clinical trials, a unifying cell sheet operation with careful detaching direction could facilitate consistent, ordered and reproducible production. Strategies to harvest cell sheets along a predetermined direction are significant to address this issue but still in the infancy.

A PEDOT film with gradient thickness was designed to provide a gradient photothermal field for locally dissolving a type I collagen layer. Type I collagen is comprised of cross-linked tri-helical peptides and forms a gel at relatively low temperature, which promptly dissolves on heating beyond 42°C. Its dissociation rate is linearly related to temperature. Here, under a gradient photothermal field, precisely directed collagen dissolution was expected to guide detachment of the cell sheet. Intuitively, as this novel scenario provides an opportunity for precisely directing cell sheet detachment, it can be envisaged as greatly facilitating broader commercial application of next-generation cell sheet engineering.

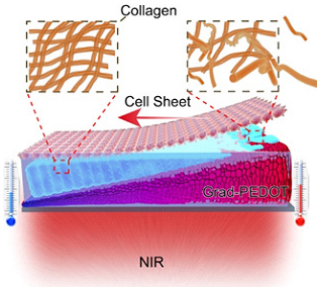

Figure 1. Scheme of gradient photothermal surface for precisely directing cell sheet detachment

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Protective effects of uncultured adipose derived stromal vascular fraction on testicular injury

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Introduction: Torsion-detorsion (T/D) induced testicular injury may lead to male subfertility and even infertility. Stem cell therapy provides an alternative to attenuate testicular injury and promote spermatogenesis. Adipose derived stromal vascular fraction (SVF) can be acquired conveniently without *in vitro* expansion, which may avoid the potential risks of microbial contamination, xenogenic nutritional sources, etc., during cell culture. In this study, we investigate the protective effects of autologous uncultured SVF on testicular injury and spermatogenesis in a rat model of T/D.

Methods: Animals were randomly divided into sham, T/D+ phosphate-buffered saline (PBS) and T/D+SVF groups (eighteen rats in each group). SVF was isolated, labeled with lipophilic fluorochrome chloromethylbenzamido dialkylcarbocyanine (CM-DiI) and transplanted into T/D testis by local injection. At 3, 7, 14 and 28 days after surgery, testicular tissue and serum samples were harvested for histopathological, immunohistochemical, Western blot and enzyme-linked immunosorbent assay.

Results: Histopathological findings demonstrated severe injury in testis with decreased Johnsen's score led by T/D, while uncultured SVF reduced testicular injury and elevated the decreased score. Injected SVF cells were mainly integrated into interstitial region and seminiferous tubules, enhanced the secretion of basic fibroblast growth factor and stem cell factor in testis, contributed to the declining level of malondialdehyde and restoration of hormonal homeostasis and then reduced the injury of Leydig cells and germ cells, as well as promoting spermatogenesis.

Conclusion: Our findings demonstrated that autologous uncultured SVF could protect testis from testicular I/R injury and promote spermatogenesis, which provide significant clinical implications for the prevention of infertility induced by testicular T/D.

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