ISSN 1313-7735

RESEARCH PEOPLE AND ACTUAL TASKS ON MULTIDISCIPLINARY SCIENCES

Proceedings of the Fifth International Conference

Publisher

Bulgarian National Multidisciplinary Scientific Network of the Professional Society for Research work

Volume 2

LOZENEC, BULGARIA
24– 28 JUNE, 2015

Organizing Committee

International Scientific Committee

ISSN 1313-7735

Printed in Bulgaria
Printing House "Angel Kunchev" University of Ruse
8, Studentska Street, 7017 Ruse, Bulgaria

PREFACE

Dear Colleagues!

The International Scientific Conference "Research People and Actual Tasks on Multidisciplinary Sciences" is fifth International Conference organized in Bulgaria with basic purpose to create the framework for the presentation, debate and publication of the valuable scientific results obtained by both the young members.

United by the idea of Multidisciplinary Sciences, the researchers and faculty will report the results of their research. Thus, the scientists will contribute is to promote exchange of research results, scientific ideas and their practical implementation and development work in the various disciplines.

We hope this meeting will initiate new joint research projects, new friendships. We owe special thanks to all participants, and especially to the supporting organizations.

Chief Editor
Assoc. Prof. Dr. Atanas Atanasov

CONTENTS VOLUME 2

Social and human sciences. Economic sciences. Ecology and Environment protection, Land & Water Use and Environment, Physics, Chemistry, Biology, Computer Systems and Technologies

OXIDATIVE STRESS AND HISTOPATHOLOGICAL STUDIES AFTER IMPLANTATION OF MODIFIED CALCIUM PHOSPHATE CEMENTS IN RAT CALVARIA

P. Dimitrov, M. Gabrashanska, V. Nanev, I. Vladov, N. Tsocheva-Gaytandzhieva, M. Alexandrov, D. Rabadjieva

Abstract: *The present study was carried out on the biochemical indices (oxidant/ antioxidant status) and histological response after implantation of modified cements based on DCPA and TTCP as solid phase and tartaric or ascorbic acids as liquid phase in rat calvarial defects. Three groups of rats were used in the experiment: 1st gr. - control (received a critical size skull defect, CSD, without any implants, 2nd gr. – with CSD and implanted material 1 and 3rd gr. – with CSD and implanted material 2. Biochemical and histological studies were done after 2 months of implantation. Serum was analyzed for free radical index contents MDA, SOD, GPx and GSH. Quantitative tissue response towards the implants was histologically investigated. MDA level was higher in the control group compared to the rest groups. No changes in the enzyme activities and GSH content were observed among the groups. No signs of inflammation were noted independently from the scaffolds 2 months after implantation. Evidence is provided in our study for good biocompatibility of newly biomaterials. Moreover, the suitability of them for other kinds of bone defects has to be proven in further experimental studies.*

Key words: *bone implants, MDA, SOD, GPx, GSH*

INTRODUCTION

During the last decades a variety of biomaterials have been used for the fabrication of orthopedic and dental implants. They serve as matrices for tissue formation and thus should fill multiple roles including mechanical strength, biodegradability and biocompatibility. Several attempts have been aimed to modify implant composition and morphology to optimize implant-to-bone contact and improve integration [5].

Calcium phosphate cements (CPC) are considered as a second generation of bioactive materials for bone regeneration. Their main advantages include the ability for in vivo self-setting, good osteoconductive and partly osteoinductive properties, good processing, low price, etc. Their drawbacks include low mechanical strength, not well-defined micro- and macroporosity that are necessary for the formation of interconnected pores [4].

The aim of the present study was to carry out a comprehensive safety evaluation of a newly developed modified cements based on dicalcium phosphate anhydrous (DCPA) and tetracalcium phosphate (TTCP) as a solid phase and tartaric or ascorbic acids as a liquid phase. For this reason serum oxidative/ antioxidant status (malondialdehyde, MDA, superoxide dismutase, SOD, glutathione peroxidase (GPx) and glutathione (GSH) as well as histological studies were done in rats with calvarial defects. MDA is the biomarker of lipid peroxidation and the enzymes SOD and GPx are the primary step of the defense mechanism in the antioxidant system against oxidative stress [2]. The second line of defense includes the non-enzymatic radical scavenger GSH, which scavenges residual free radicals resulting from oxidative metabolism and escaping decomposition by the antioxidant enzymes [3].

MATERIAL AND METHODS

Modified cements preparation

Solid phases: Dicalcium phosphate dehydrate (DCPD) and CaCO3 (Sigma Aldrich, for analysis) were used as raw materials. Firstly DCPD was thermal dehydrated at 200°C and powder DCPA was obtained. The last one was used not only as a solid phase for the cement preparation but also as a raw material for TTCP preparation. For this an equimolar

mixture of DCPA and CaCO3 (Sigma Aldrich, for analysis) was prepared and sintered at 1500°C for 5 h.

An equimolar powder mixture of the both solid phases DCPA and TTCP was prepared and ball milled for 5 h in order to activate the particles surface. The solid mixture was fractioned and powder size < 28 μm was used in the experiments.

Liquid phases: Tartaric or ascorbic acids (Merck, analytical grade quality) (18% solutions) were used as a liquid phase for the cements preparation.

Modifiers: Xanthan gum (2% solution) and glycerin (5% solution) were used as medium and manipulation modifiers.

The solid/liquid ratio was 2.6 g/ml.

The cement prepared with tartaric acid is further denoted as CPCt, while this one with ascorbic acid – CPCa.

The resulting samples were characterized by initial and final setting time (Vicat needle method [1] and XRD analysis (Bruker D8 advance XRD apparatus).

Animal models. Eight-week old male rats weighed approximately 350 g were used in the experiments. The rats were allocated to three experimental groups. Animals in the control group received a critical size skull defect (CSD) with no scaffold implantation. The rest two groups received implants as follows CPCt /group 1/ and CPCa /group 2/.

General anesthesia was given. To create a CSD in the skull the head was shaved and cleaned with antiseptic. A lateral longitudinal incision over the head was made under aseptic conditions. The skull cortex was drilled and a calvarial bone defect 1, 8 mm wide and 6 mm long was created. The biomaterials were implanted into the defect zone and their position was checked. The wound was then closed with continuous subcutaneous stiches. Animals had free access to food and water and were monitored daily in the postoperative period for any complications or abnormal behavior.

After 2 months the animals were sacrificed with over dose of pentobarbital. Blood was collected from the abdominal aorta in collection tubes for serum.

Histology. Immediately after death, the head was cut off at the atlanto-occipital joint, and immersed in 10% neutral buffered formalin for a week. Then the mandible and all surrounding soft tissue were removed and the remaining cranium was cut at two transversal segments 3-4 mm wide at the calvarias implant levels using a stomatological Axis Diamond Disc (Fig. 1). The obtained transversal segments were immersed in 10% formic acid for demineralization which lasted at least three weeks at 37°C. The acid solution was changed every 24 hours. The demineralization process was monitored daily, testing the specimens with a needle. Finally the specimens were each submitted to a neutralization process with PBS pH 7, 2 (3 changes in 24 hour intervals) and dehydrated in ethanol. Than the materials were processed in chloroform (3 changes in 24 hour intervals until the implants were totally dissolve), embedded in paraffin, cut at 6-8 m sections and stained with hematoxylin-eosin according to the standard histological technique. The histological evaluation of tissue response against the implants was carried out of Leica DM 5000B microscope.

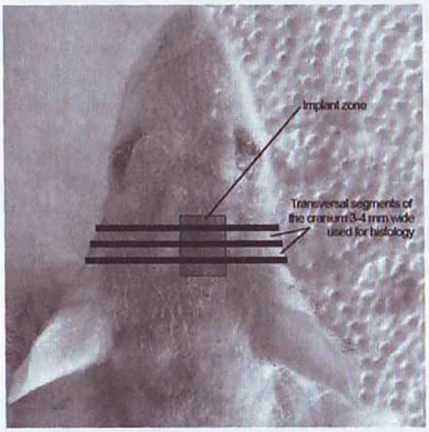

Fig.1. Schematic rat head with a calvaria defect covered with implant. The image shows the implant levels where two transversal segments 3-4 mm wide were obtained for histology.

Biochemical indices MDA, SOD, GPx and GSH were measured as indicators of free radical burden (Table 1). No significant differences between the treatment group and the group with empty defect were observed for glutathione, SOD and GPx. MDA was significantly increased in the control group compared to the rest groups. These differences may be related to chemical structure of the two implants.

Table 1. Oxidant/ antioxidant parameters

	MDA μmol/l	GSH μmol/l	SOD U/mg protein	GPx U/mg protein
Control group	30,24± 2,15	6,6± 0,9	0,25± 0,06	10,1± 0,2
Group 1	21,5± 3,18	5,2± 0,4	0,19± 0,04	9,6± 1,5
Group 2	25,6± 1,94	5,9± 0,7	0,2± 0,03	10,4± 1,7

This study was performed to evaluate the tissue reaction to newly synthetized implants. The new biomaterials were well tolerated by the host organisms. They did not evoke adverse reactions such as long-term reactions. Histological examination demonstrated that the new materials support bone formation. The lack of visible inflammatory complication at the implantation site, body temperature and a histological examination provided no signs of a systemic inflammatory reaction. These findings were supported by Indicators for free radicals (GSH, SOD, GPx) which were associated with inflammation were not increased, compared to the control group. The shown data revealed an increase in MDA, the biomarker of lipid peroxidation that is the additional indicator of oxidative injury. In conclusion the MDA levels were increased after 2 months, but did not accompany with changes of SOD, GPx activity and GST level. The difference between antioxidants and MDA level suggested that cellular repair system of oxidative damage was able to reduce ROS rapidly, but could not decrease MDA level significantly after implantation. Oxidative stress plays a very important role in the complications of ceramic implants. This is in line with available reports in this field [6, 8].These results indicate that the new materials have less effect on the redox stat of rats with empty calvarial defect. There were differences between the effects of both materials on histological and biochemical responses in the host- rats.

CONCLUSION AND FUTURE WORK

The biological efficacy of the newly implants is well expressed in a calvarial defect rat model. Our study gave essential information for the design of novel bioceramics.

The histological examinations performed clearly indicate that implants used in the present study successfully could be applied for covering of small skullcap defects. Indicative for that were the lack of foreign body reaction and inflammation around the implants, as well as, the good integration of implants with the surrounding tissues.

No signs of inflammation were noted independently from the scaffolds 2 months after implantation. Evidence is provided in our study for good biocompatibility of newly biomaterials. Moreover, the suitability of them for other kinds of bone defects has to be proven in further experimental studies.

ACKNOWLEDGEMENTS: This work was financially supported by the Bulgarian Ministry of Education and Science under Project DFNI T02-5/2014.

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