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Contributions of chemistry and material sciences to the translational research: examples of the current research

Translational research involves basic research, patient-oriented clinical research and population related research targeting to improve sustainably the public-health. New approaches to materials, preventions of disease, diagnostics and therapies access clinical trials in order to enhance steadily the therapeutic success for the affected patients. Essential requirements are the efficient partnership of physicians, researches in natural, biomedical and material sciences, and the medical device and pharmaceutical industry as well. Briefly outlined examples of the collaboration between the natural sciences in Tomsk and Muenster (Germany) and an orthopedic clinic and a radiation therapeutic/oncologic department (Muenster, Germany) demonstrate the contribution of chemistry and material sciences to the translational research.

Keywords: *translational research; examples of the collaboration; biomaterials; "from bench to bedside and back again".*

Introduction

According the definition of Translational Research published by the US National Institute of Health (NIH) on July 26, 2002, translational research is the process of applying ideas, insights, and discoveries generated through basic scientific inquiry to the treatment or prevention of human disease [1]. The discoveries in molecular and cell biology in the last half century such as the solved decodification of the human genome in 2003 [2–5] have aroused among scientists and the public too, high hopes for a quick translation from biomolecular and genome research into medical practice. By 2012, the NIH expects to establish 60 centers in US, with an annual funding commitment of $500 million. "Science" has launched a new magazine in October of 2009 called Science Translational Medicine competing with journals such as the American Journal of Translational Research and the Journal of Translational Medicine. The Seventh Framework Program (FP7) of EU provided a budget of over € 50 billion (2007–2013), of which 12% was targeted to health [6] emphasizing projects related to translational research in infectious diseases, cancer, cardiovascular disease, diabetes, obesity, and rare diseases. According the Degree of the President of the Russian Federation no. 598 (May 7th, 2012) on the improvement of the national

health care system [7, 8] clusters and centers of translational medicine have been founded in recent years in Saint Petersburg, Moscow, Kazan and Tomsk (SSMU and ТГУ). Meanwhile manifold translational research cluster has been established worldwide.

Translational research bridges clinical and basic research characterized by the multidisci-plinary collaboration in order to develop high specific diagnostics, novel and efficient therapies and the prevention of disease. With respect the biomedical and the stem cell engineering inspired regenerative medicine, the increased knowledge on biomaterials, and the high standards of optomechanics and software as well, the modern medicine faces both, high sophisticated challenges and new horizons in the multidisciplinary research. Investigations on the pathophysiology of diseases and the mechanisms of the biomolecular processes, and the development of specific pharmaceuticals, appropriate biomaterials for tissue scaffolds or drug delivery systems and adequate medical devices have to be closely executed in partnership with the clinical research, and the related industry as well. Translational research challenges investigators to turn away from the traditional training. The current state of research needs to build bridges between clinical and basic researchers to generate jointly and translate findings from bench to bedside and back again [3].

Bilateral translational cooperation in medical technology at a glance

The laboratory of transmission cell-like and molecular biomedicine and the laboratory of catalysis of the National Research Tomsk State University have installed collaborations with scientific institutions in Germany, respectively with the Institute of Transfusion Medicine and Immunology of the Medical Faculty Mannheim, the Westphalian Wilhelms University and the company Marcotech oHG in Muenster. Mrs. Prof. Dr. Julia Kzhyshkowska, Mannheim, acts scientific supervisor at the TSU Tomsk and is involved in the current research concerning the development of novel biomaterials. Mr. Prof. Dr. Lothar Heinrich, Muenster, has provided lectures on the modern medical technology and related scientific issues.

In the frame of the current research on biomaterials two young scientists of the TSU were delegated to the Institute of Transfusion Medicine and Immunology (Mannheim, Germany) in 2015. Supplemen-tary experiments were executed in Muenster (Germany) in the laboratories of the university and the company Marcotech oHG. They presented successfully a poster at the Annual Meeting of the German Society of Biomaterial, November 12–14, 2015, Freiburg (Germany).

Fig. 1. Prof. Kurzina Irina, Lytkina Daria and Shapovalova Yelena (TSU Tomsk) at the conference of the German Society of Biomaterials, Freiburg (Germany), November 12–14, 2015

Fig. 2. Lytkina Daria (TSU Tomsk) investigates biomaterial surfaces using Atomic Force Microscopy at a laboratory of physical chemistry of the University of Muenster (Germany) assisted by Dr. Christian Herkt-Bruns, December 16, 2015

The cooperation of TSU Tomsk with the German partners contributes efficiently to the translational efforts, and enlarges the multidisciplinary network as well. The educational training in medical technology at the Westphalian Wilhelms University involves practical trainings of young chemists and students in the neighbored clinics. In addition to lectures and seminars in medical technology the students of the natural faculty are welcome to watch surgeries, or to visit departments such as dialysis, orthopedic workshop, radiotherapy and oncology. Close cooperation in clinical and natural sciences is established between the Clinic of General Orthopedics and Tumor Orthopedics, the Clinic of Radiation Therapy and Oncology and the Department of Medical Microbiology. In addition to the joint education manifold joint projects have been successfully executed, and young scientists of chemistry and material sciences are involved. Concerning the translational research, the paradigm “from bench to bedside and back again” has been implemented. No doubt, the translational network has to be carefully extended by biomolecular scientists and cell technology avoiding any loss of efficacy caused by an organizational complexity.

Young scientists of TSU are invited to stay in Germany to be involved in the existing network. Scientific issues coming out of the clinical research will be transferred to natural science and vice versa.

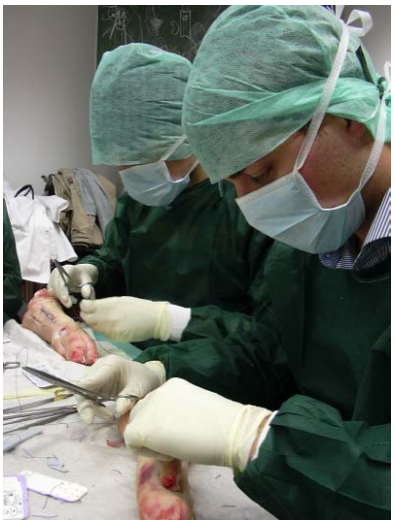


Fig. 3. Students of the Faculty of Chemistry (Muenster) learn together with medical candidates stitching wounds using different types of surgical suture material

Examples of translational research and the transfer of findings
Joint research on orthopedic materials

The distal femur is a common anatomic location for primary and metastatic bone tumors. These malignant tumors traditionally were treated with resection, arthrodesis or amputation of the extremity. This reconstruction of the skeletal system improves significantly the survival rates among the patient. Custom-made prosthesis are used to adjust individually stem length or diameter. The medical device industry supplies also expandable prosthesis which are used in patients younger than 12 years [9].

In the case of massive osteosarcoma the Clinic of General Orthopedics and Tumor Orthopedics, Muenster, has successfully implanted MUTARS®-tumor endoprosthesis [10] which has jointly developed with the company IMPLANTCAST (Germany) [11]. The modular construction makes possible the patient related adjustment. Loading materials are $TiAl_4V_6$ and CoCrMo alloys. The modules are coated with silver in order to avoid postoperative biofilms [12]. The prosthesis stem is coated with hydroxylapatite in the case of cement free implantation. UHMWPE inlays, alternatively highly crosslinked XLPE or HXLPE, in the knee joint provides the adequate tribology to the polished TiN coated CoCrMo joint ball of the distal femur stem. The joint is stabilized by a cylinder like lock system of PEEK. A woven attachment tube of PET is used for the refixation of the soft and muscular tissue and tendons and for the reconstruction of capsular structures.

Limb salvage with the multicomponent MUTARS® endoprosthesis has been proved regarding good functional results and low rates of periprosthetic infec-

tions. But complications cannot be excluded such as wear or break of components, especially polymers, aseptic loosening, perioperative infections, and septic loosening, as well as the local recurrence of tumor.

Fig. 4. Components of an explanted knee tumor endoprosthesis (after wear time of 3 years)

The collaboration between the clinic and the chemistry and material science was focused on

– indications of any biofilm forming on the metal and polymer surfaces or the PET filaments,

– the postoperative change of the silver surface color to brown or black, and the biochemical interpretation of that effect,

– causes of failures such as breaks or cracks,

– effects on the silver coating of different antiseptics as perioperative lavages in order to avoid adverse surface reactions,

– long time stability of the woven attachment tube, and the tissue integration as well.

The continuous collaboration has been started several years ago, and young scientist and graduate students of natural and material sciences are actively involved. They are guests at orthopedic surgeries and assist in sampling under sterile conditions. The young candidates are integrated in analytical tasks and experiments, as well as in the related current disputation with the clinical researchers. Examples as following illustrate the current collaboration between clinical research and natural sciences.

10 explanted prosthesis were investigated on microbial contaminations and biofilms. Neither MALDI-TOF-MS analysis (*matrix aided laser desorption/ionization; time-of-flight mass spectroscopy*) nor SEM (*scanning electron microscopy*) could indicate any bacteria on the metallic and polymer components. Several areas were covered by adhering denaturated proteins:

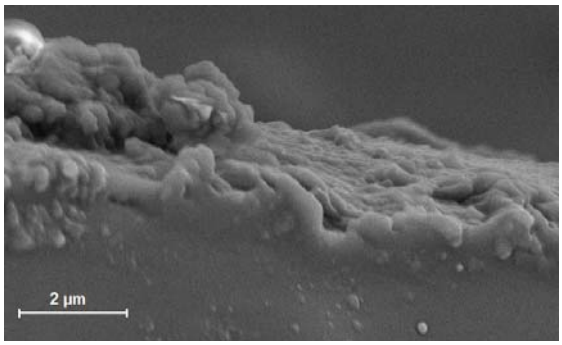


Fig. 5. Typical SEM micrograph of deposits on components of explanted endoprosthesis. Both, the small sizes of the species and MALDI-TOF analysis exclude any bacterial provenance

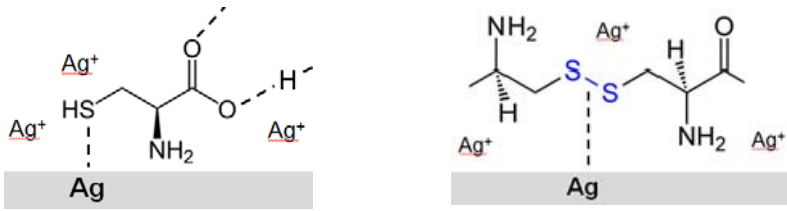
This result corresponds with the clinical practice, respectively the reduction of postoperative infections of about 30% cases to 5% after the use of silver coated tumor endoprosthesis [10, 12].

The explanted silver coated modules show always changes of colors which differ from yellow to brown or sometimes black. Energy dispersive X-ray spectroscopy (EDX) indicates forming sulfur and nitrogen containing surface compounds.

Fig. 6. Silver coated prosthesis module before implantation

Fig. 7. Explanted silver coated module after a wear period of 3 years, tumor endoprosthesis was removed due to an aseptic loosening

The etiology of the individual differences of coloring is unknown until now. The extent of silver that can be released into biological fluids from the silver coating depends on the protein characteristics, adsorbed layer properties, formation of silver-protein complexes as well as concentrations of proteins in the solution. WANG et al [13] postulate an enhanced release of silver in the presence of bovine serum albumin (BSA) attributed to surface complexation between BSA and silver:



As hypothesis of the mechanisms may be the interactions of silver with sulfur containing cystin and cysteine protein sequences supporting the adhesion. Denaturation and silver catalyzed degradation are followed forming soluble or adherent silver complexes, and black coloring insoluble Ag_2S.

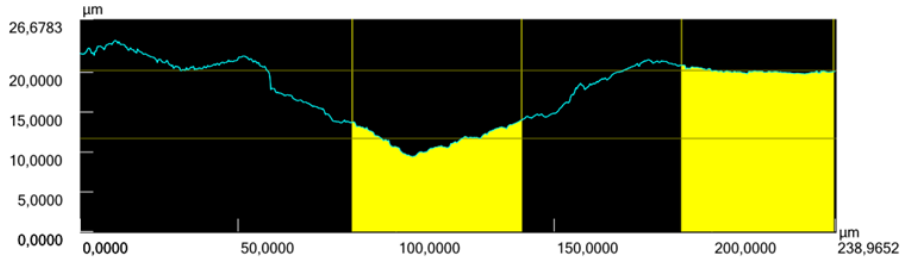


Fig. 8. The measurement of the silver depth profile using LSM (laser-scanning-microscopy; KEYENCE, VK-X200) discloses the erosion of the coating by loss of silver within the dark colored areas

Clinical studies have shown that the silver coated tumor endoprosthesis caused postoperative silver concentration in blood can arise to 0,08 ppm [14]. Titanium and silver can be found in the bone-implant interfaces, about 700 ppb Ti and 800 ppm Ag. Therefore, neither systemic nor local argyrosis (blue coloring) have not been considered. In fact, the chemical structures of the complexes are not known yet, as well as their toxicology, side effects, accumulation in tissues and elimination by urine. These open questions are issues of the current joint research.

In addition to the systemic prophylaxis against surgery associated infections the prosthesis and open wounds have to be rinsed out with antiseptic liquids. The effects on silver of several typical lavages were investigated. It could be demonstrated that the use of BETAISODONA®, solutions of polyvinyl pyrrolidone-iodine-complex, or any iodine containing antiseptic are unfavorable due to the forming of surface silver complexes and compounds which cause undesired side effects with proteins. Translated these results to the clinical practice only H_2O_2, LAVASEPT®, PRONTOSAN® (solutions of polyhexanide) or formaldehyde are used as perioperative antiseptic lavages.

The attachment tube has been developed for the refixation of soft tissue and tendons and for the reconstruction of capsular structures.

Fig. 9. The woven PET-attachment tube fixed by bioresistant strings envelops the endoprosthesis

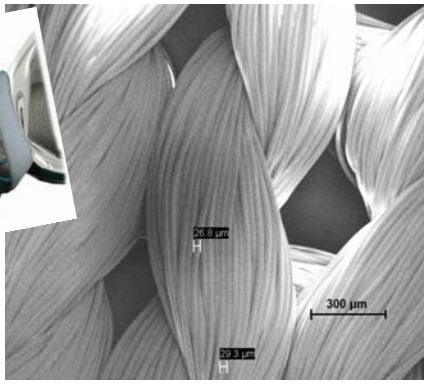


Fig. 10. Each string consists of about 185 parallel running PET-monofile, diameters of 25 µm

The open structure of the fabric allows the postoperative tissue integration into the attachment tube, respectively the refixation of muscular and further soft tissue. Risks of subluxation are prevented supporting the successful application of the tumor prosthesis.

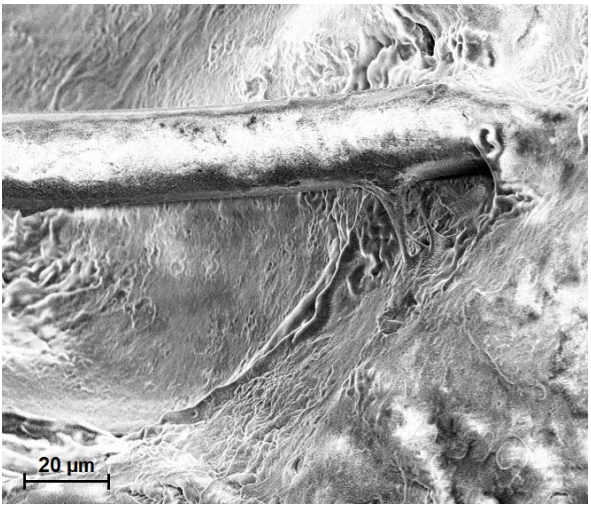


Fig. 11. SEM micrograph of a closely tissue surrounded PET-monofile (one example of an explanted attachment tube)

It has been shown by investigation of more than 10 explanted samples on microbial species or biofilms (MALDI-TOF-MS, SEM and cultivations after microbial impression technique) that the attachment tube was free of any biofilms. One case of small amounts of *Serratia marcescens* was indicated by MALDI-TOF-MS, perhaps caused by endogenous infiltration.

With respect to these results and the clinical experiences as well, the parallel running monofiles within the PET-fabric achieve both, soft properties and long-time mechanical stability. The intensity of movements and the frictions between the monofiles destroy obviously emerging biofilms.

Drug delivery systems for IORT

A joint research on a local drug delivery system has been established with the Clinic of Radiation Therapy and Oncology at the Westphalian Wilhelms University Muenster in order to protect the healthy tissue against *reactive oxygen radicals* (ROS), which are secondarily generated by ionizing rays. *Intraoperative Radiation Therapy* (IORT) such as the *external beam radiation therapy* (EBRT), the *high dose radiation* brachy therapy (HDR) or the novel *Intrabeam® Photon Radiosurgery System* (PRS) apply X- or gamma-rays [15]. The biomechanical transport processes and the biomolecular cellular mechanisms have to be understood for the design of appropriate antioxidants loaded nano-containers, as well as to design an useful drug delivery device for clinical trials.

Typical risks and serious acute effects of IORT are e.g. erythema, postoperative ulcer, and late side effects can be chronic inflammation, perioperative infections, injuring neighbored organs or triggering malfunctions or recurrence [16, 17].

The demand of a locally applied antioxidant delivery system in combination with antiseptics has been indicated by clinical experiences. The idea is that a gel like overlay will be loaded with water solubilized antioxidants in order to deliver the antioxidants in the tissue [18]. The delivery system is positioned around the radiation target (skin, wound bed) carved to an appropriate form. With respect to the fractionated radio-treatments the overlay has to be easily removed. With respect to the barrier properties of the upper skin layer (*stratum corneum*) the antioxidant loaded nano-container of the highest penetration rate has to be selected. The joint research involves the transdermal penetration, the transport within deeper layers such as epidermis or connective tissue, and the biomolecular processes to scavenging ROS as well.

The following drawing illustrates the different transport routes across the *stratum corneum*. In general, lipophilic drugs pass the death corneocytes. The intercellular path is blocked by desmosomes consisting of lipids which are secreted from keratinocytes during the mutation to corneocytes. Physical stimulations such as ultrasound or electrophoresis can destroy these desmosomes, but also penetration enhances such as solvents, surfactants, fatty acids can open the lipophilic transport route.

Within any soft connective tissue, dermis or epidermis the intercellular transport can be described with the FICK´s laws in combination with cellular uptake and elimination via blood capillaries. The nanocontainers interact with the cell membranes and access the cytosol by endocytosis. The liposomes or micelles dissipate and release the antioxidants in order to deactivate ROS.

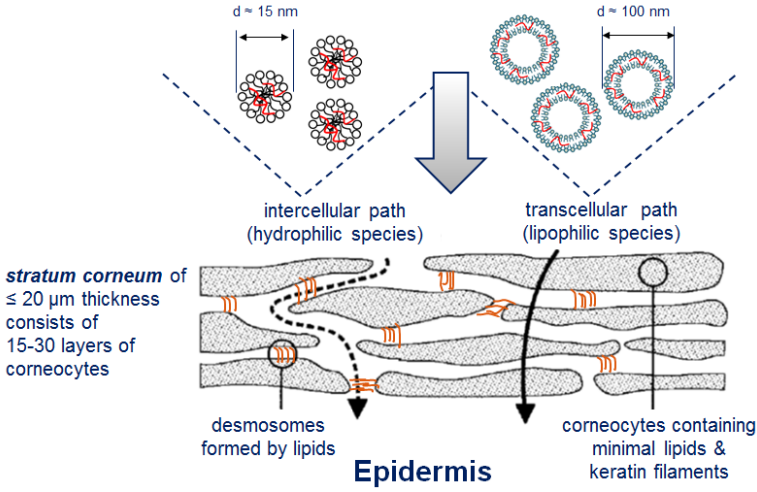


Fig. 12. Scheme of the transport routes across the barrier stratum corneum; sizes of the drug loaded micelles and liposomes should be smaller than 1 µm to pass the hydrophilic intercellular spaces

The treatment with antioxidants requires a higher protective effect to healthy tissue than to the remaining tumor cells. A test series with antioxidants has been started with D-α-tocopherol and a tocotrienol rich raffinate. In-vitro investigations on the antioxidants´cell toxicity and irradiation effects on the cell survival fractions at human fibroblast cell line HaCaT and hormone receptor positive breast cancer cell line MCF-7 have shown that under the exposure of the antioxidant loaded nanocontainers the tocotrienols containing Palm-TRF provides a higher radiation protecting efficacy to healthy cells than to cancer cells such as MCF-7.

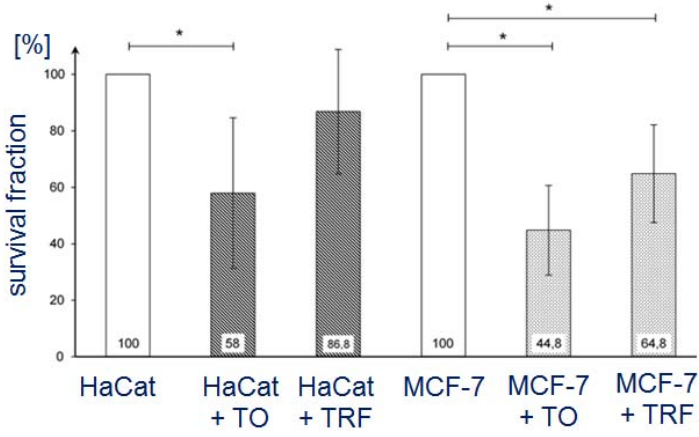


Fig. 13. Survival fractions of HaCat and MCF-7 cells after X-ray radiation (dose: 2 Gy) under exposure of D-α-tocopherol (TO) and tocotrienol rich raffinate (TRF)

These preliminary results motivate to continue the search for antioxidants of higher radioprotecting selectivity to healthy cells. Therefore further bioactive antioxidants will be involved in the future investigations such as the use of isolated tocotrienols, hydroxytyrosol, L-ascorbic acid etc., as well as alternative techniques of carrying (e.g. complexation with cyclodextrins). Furthermore, a simplified model of the transport across skin and open tissues has been developed [20] and will be optimized by pharmacokinetic studies. The design of the appropriate wound dressing or overlay will be elaborated together with the clinic and an experienced company.

In contrast to the envisaged locally targeted effect of the antioxidants, the transdermal drug delivery is already established for the systemic medication with nicotine, chemotherapeutics etc. [21, 22]. With respect to the broad medical application, the currently executed collaboration provides a huge potential for future translational research.

Outlook and conclusion

The presented examples of translational research demonstrate that chemical and material science can efficiently contribute to advantages in the clinical medicine. Bridging research of clinical and natural science should be understood that manifold modern sciences has to be involved, e.g. biomolecular and regenerative medicine, genetics, pharmaceutical research and natural sciences such as chemistry and material sciences. The creation of an adequate network of scientific excellence is one point of view, the efficacy of collaboration and knowledge transfer make necessary a well-functioning organizational structure and management. Translational networks are marked by intrinsic dynamics and the ability to involve external partners depending on the scientific issues.

The dream to catch new horizons in medicine stimulates the translational research. However joint research which promises success in the near future, motivate the partners to continue and intensify the collaboration.

Training of young scientists in translational thinking and acting should be started in an early phase of the education in order to become familiar in bridging natural and medical issues and to destroy barriers of communication. The upcoming and recognizable challenges in medicine require multidisciplinary competence, respectively the ability to collaborate and communicate with partners of best excellence.

References

1. NINDS Exploratory/developmental projects in translational research, program, NIH, announcement number: PAR-02-138, July 26, 2002.
2. Gregory S.G., Barlow K.F. The DNA sequence and biological annotation of human chromosome 1 / S.G. Gregory, K.F. Barlow u.a. // Nature. 2006. Vol. 441. P. 315–321.
3. Marincola M.J. Editorial: Translational Medicine: A two-way road // J. Transl. Med. 2003. Vol. 1. P. 1.

4. Zerhouni E. Medicine. The NIH Roadmap // Science. 2003. Vol. 302. P. 63–72.
5. Lee W.-H., Languino L.R., Hung M.-C., Dubinett S.M., Iczkowski K.A., Wang D. The launch of the American Journal of Translational Research // Am. J. Transl. Res. 2009. Vol. 1. P. 1.
6. The E.U. total health budget for framework 7 Available from: http://ec.europa.eu/research/fp7/index_en.cfm?pg=health.
7. Ukaz Prezidenta RF ot 7 maya 2012 g. N 598 "O sovershenstvovanii gosudarstvenniy politiki v sphere zdravookhraneniya".
8. Pravitelstvo Rossiiskoi Phederazii, Rasporyazhenie ot 24 dekabrya 2012 g. no 2511-r.
9. Bickels J. et al. Distal femur resection with endoprosthetic reconstruction // Clin Orthop Relat Res. 2002. Vol. 400. P. 225–235.
10. Gosheger G., Gebert C., Ahrens H., Streitbuerger A., Winkelmann W., Hardes J. Endoprosthetic reconstruction in 250 patients with sarcoma // Clin Orthop Relat Res. 2006. Vol. 450. P. 164–171.
11. Website of IMPLANTCAST GmbH (Russian): www.implantcast.de/index.php?lang=ru.
12. Hardes J., von Eiff C., Streitbuerger A., Balke M., Budey T., Heinrichs M.P., Hauschild G., Ahrens H. Reduction of periprosthetic infection with silver-coated megaprostheses in patients with bone sarcoma // J. Surg Oncol. 2010. Vol. 101. Is. 5. P. 389–390.
13. Wang X., Herting G., Wallinder IO., Blomberg E. Adsorption of bovine serum albumin on silver surfaces enhances the release of silver at pH neutral conditions // Chem Phys. 2015. Vol. 17(28). P. 18524–18534.
14. Hussmann B. et al. Measurement of the Silver Ion Concentration in Wound Fluids after Implantation of Silver-Coated Megaprostheses: Correlation with the Clinical Outcome / B. Hussmann et al. // BioMed Research International. 2013. article ID 763096, 11 pages.
15. URL: http://dx.doi.org/10.1155/2013/763096.
16. Beroukas E. et al. Intraoperative electron beam radiotherapy followed by moderate doses of external beam radiotherapy in the treatment of resected soft tissue sarcomas of the extremities // J. BUON. 2004. Vol. 9. Is. 4. P. 391–398.
17. Singh J., Kaur M. Current Advances and Stretegies for Reducing the Side Effects of Radiation Therapies used for Various Types of Cancers // Int J Appl Biol and Pharm Tech. 2012. Vol. 3. Is. 1. P. 184–199.
18. Jolyon H., Hendry J.H., Jeremi B., Zubizarreta E.H. Normal tissue complications after radiation therapy, Rev Panam Salud Publica // Pan Am J Public Health. 2006. Vol. 20(2/3). P. 151–160.
19. Kumar B., Jha M.N., Bedford J.S., Prasad K.N. D-alpha-tocopheryl succinate (vitamin E) enhances radiation-induced chromosomal damage levels in human cancer cells, but reduces it in normal cells // J. Am. College of Nutr. 2002. Vol. 21. Is. 4. P. 339–343.
20. Pang Z., Han C. Review on Transdermal Drug Delivery Systems // J. Pharma & Drug Dev. 2014. Vol 2. Is. 4. P. 1–10.
21. Bhowmick M., Sengodan T. Mechanisms, kinetics and mathematical modelling of transdermal permeation – an updated review // Int. J. Res. Dev. Pharm. L. Sci. 2013. Vol. 2. Is. 6. P. 636–641.
22. Sharma A., Saini S., Rana A. C. Transdermal Drug Delivery System: A Review // Int. J. Res. Pharm. and Biomed. Sc. 2013. Vol. 4(1). P. 286–292.

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