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ADVANCED MATERIALS IN ORAL PHYSIOLOGY AND PATHOPHYSIOLOGY RESEARCH

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Abstract. With the rapid development of nanotechnology, in recent years, the application of advanced materials in dentistry is widely researched. Some scientists agree that nanomaterials might be very useful in caries prevention and treatment, as well as in reconstructive dentistry and bone regeneration. Some of compounds currently in focus include titanium, silver nanoparticles, chitosan, calcium phosphate, nano-hydroxyapatite, and nanoceramics. Certain materials may have unique physical and chemical properties that make them applicable in design of dental implants. Other nano-based systems are suitable for experimental research in fundamental medical/dental sciences such as cell biology and histology. However, some important issues, such as local and systemic toxicity of certain nanomaterials need to be resolved before they can be more widely used in clinical practice. This concise review focuses on recent research on potential application of these advanced compounds in oral physiology, pathophysiology and clinical disciplines.

1. INTRODUCTION

Over the last several decades, nanotechnology has revolutionized many aspects of biological and medical research. With the development of novel, advanced nanomaterials with unique physical properties and applications, researchers were able to propose innovative diagnostic and therapeutic strategies in many fundamental and clinical medical areas. Dentistry is no exception, where advanced materials were suggested to be applicable in caries prevention and treatment, dental biomimetics, anaesthesia, as well as periodontal drug delivery. Various studies have also been performed to investigate the use of nanomaterials as biosensors of active compounds in oral fluid, and as potential inductors of osteogenesis. These and other applications are promising, and still in initial stages of development, so it is estimated that in the next several years nanodentistry will become a rapidly growing multidisciplinary area which will significantly contribute to our knowledge and practices in stomatology [1-3].

Particularly important is the fact that in fundamental medicine, nanoparticles can be used both as biosensor and chemical delivery systems in virtually all tissues. This opens new possibilities for the research of normal, physiological processes occurring in oral cavity environment, as well as for investigation of pathophysiological basis of oral diseases. For example, it is possible that in the future, thanks to novel technologies in advanced material design, we will be able to better comprehend the physiology of saliva production, and immunomodulatory processes occurring in saliva. Also some aspects regarding the development of

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periodontal disease could be clarified using these novel research approaches [1-5].

In this concise review, we focus on the recently published data on potential nanomaterial application in oral physiology, pathophysiology and related disciplines. We also present suggestions and ideas for future research in these fields, and discuss potential limitations and challenges that need to be resolved before nanomaterials can be introduced in everyday dental practice. Local and systemic toxicity of certain nanomaterials, as well as immunomodulatory effects of some types of nanoparticles are also discussed.

2. NANOMATERIALS AND CARIES RESEARCH, PREVENTION AND TREATMENT

Dental caries (cavity) represents the process of teeth degradation as the result of bacterial action. Some types of bacteria normally present in the oral cavity such as Streptococcus mutans and Lactobacillus species, process simple sugars present in the food, and produce relatively large amount of lactic acid. Low pH caused by the lactic acid, leads to the breakdown of enamel and dentin and formation of cavity. Sometimes, at first, bacteria form a thin layer, or biofilm, which is in literature commonly referred to as plaque [6-8].

It is thought that in the future nanomaterials could be successfully applied both for caries prevention and treatment, as well as better understanding of principles of caries formation [1-3,9,10]. For example, various nano-based systems have been created in order to increase enamel mineralization and reduce teeth erosion. Many of these systems contain nano-hydroxyapatite as the main active component [11-14]. For example, nanocrystals based on calcium fluorinated hydroxyapatite may particularly be useful for enamel and dental recovery

Some nanomaterials have known antimicrobial properties which could make them good candidates for cavity prevention. Silver nanoparticles, in colloidal solutions and in certain concentrations have significant antibacterial effect, and in some clinical disciplines, they are applicable as an addition to wound dressings [15]. Colloidal silver in some countries is available as over-the-counter commercial product, often used as dietary supplement, although its beneficial effects when taken in this form are not thoroughly investigated, and sometimes disputed. There are indications however that primarily due to its antiseptic properties, silver nanoparticles may be applicable in oral medicine for preventive purposes, and for maintaining oral hygiene. However, before this application may be introduced in common dental practice, potential toxicity of silver nanoparticles on human cells and tissues must be investigated [15-17].

Antimicrobial effects of other nanosystems has also been evaluated. For example, caseinphosphopeptides – stabilized calcium phosphate nanoparticles can also be successfully used for dental caries prevention. It has been suggested that these nanomaterials decrease the probability of biofilm and plaque formation on enamel by certain types of bacteria such as Streptococcus mutans [18,19]. Samprasit et al. (2015) reported that mucoadhesive electrospun nanofibre mats produced with the addition of thiolated chitosan (CS-SH) as mucoadhesive polymers may be useful for caries prophylaxis [20]. This complex material could substantially reduce bacterial growth, without inducing cytotoxicity to normal tissue. graphene/zinc oxide nanocomposite film has been found to provide significant protection against Streptococcus mutans. Kulshrestha et al. (2014) found that this film is a potentially effective and nontoxic protective agent for implant surfaces [21].

It should be noted that these nano-based systems are still under investigation, and have not yet become fully applicable in dental practice. Apart from future confirmation of their antibacterial effectiveness, additional research also needs to be performed regarding their potential local and systemic toxicity.

3. POSSIBLE APPLICATION OF ADVANCED MATERIALS IN RECONSTRUCTIVE DENTISTRY AND BONE REGENERATION

Use of nanomaterials as an integral part of implants for replacement of teeth and bone tissue is currently being investigated in numerous research laboratories [9,10]. Some of the promising compounds include, but are not limited to nanostructured hydroxyapatite and nanostructure metalloceramic coatings, as well as alumina/zirconia nanocomposites. Chlorhexidine-hexametaphosphate nanoparticles and carbon nanotubes are also potential candidates for implant incorporation and/or modification in order to increase the success rate of the operation and the postoperative healing time [22-25].

Historically, titanium has proven to be one of the best materials in implant manufacturing and placement, predominantly because of its specific inter-

actions with bone and other surrounding tissues after the surgery. Kim (2014) suggested that modification of dental implant surface by Nano Titania could be safe and beneficial for initial bine formation after the procedure [26]. Titania particles used in this study proved to be relatively nontoxic since the elute from titania-treated fixtures had no significant negative effects on cell viability.

Although titania-based materials have relatively good biocompatibility and mechanical properties, bacterial accumulation and subsequent inflammation remain one of the most important factors affecting the success rates of implant procedures in dentistry [27]. Fortunately, some surface modifications of these materials, such as the addition of silver, can substantially reduce the probability of bacterial proliferation. For example, Huang et al. (2010) reported that TaN-Ag nanocomposite coatings with different Ag contents exhibit significant antibacterial properties, particularly against Staphylococcus aureus.

In recent years, much attention has been focused on development and research of nanoceramics. It has been noted that nanoceramics have specific chemical and chemical properties which enable this material to facilitate bone forming [28-30]. Certain types of ceramic structures can enhance proliferation of osteoblasts, cells that are crucial in bone growth and remodeling.

Nanosized hydroxyapatite might have numerous application in reconstructive dentistry and related disciplines. Singh et al. (2012) demonstrated that this advanced material in bone graft in combination with bioresorbable collagen membrane is particularly useful in the treatment of intrabony periodontal defects [31]. Chen et al. (2011) described the properties of high aspect-ratio hydroxyapatite (HAP) nanofibers and suggested that incorporation of small mass fraction of these materials can be applicable in terms of improving structural/mechanical features of dental resins and dental composites [32]. Yasaei et al. (2013) indicated that hydroxyapatite nanoparticles may improve the mechanical strength of Calcium hydroxide cement as pulp-capping substance [33].

Similarly as with materials with antibacterial properties, the full effectiveness of some of the above-described systems, as well as their potential toxicity remains to be fully investigated in the future. Also, the cost and the requirement of additional resources (novel technologies for synthesis, expertise, etc.) need to be evaluated before these material can be used in clinical setting.

4. FUTURE STRATEGIES FOR APPLICATION OF NANOMATERIALS IN STOMATOLOGY: FOCUS ON POTENTIAL TOXICITY

It is estimated that in the future, many important issues will have to be addressed before nanomaterials become an integral part of everyday stomatological practice. The most important challenge that researchers are today faced with remains potential toxicity of nanomaterials on human cells and tissues. Several studies have already been conducted on the potential toxic effects of metallic nanoparticles, such as silver, gold, platinum, zinc, titanium, and iron.

Unfortunately, most of these studies have only been done on individual cells and cell cultures. Any biological/toxic phenomenon observed in individual cells does not necessarily have to be present in the living tissue. In tissues, cells are surrounded by a specific microenvironment with many different potentially confounding factors that may influence the previously observed relationship in *in vitro* conditions. Various hormones, cytokines, growth factors and chemical mediators may in some circumstances increase or reduce the toxicity of nanoparticles.

Before a specific nanomaterial is considered for application in stomatological practice, in animal experimental models, we will have to determine its median lethal dose, LD_{50}, or the minimal dose required to kill 50% of the tested animal sample. For many of today commercially available nanomaterials, this important toxicity parameter has not yet been defined. Even with known LD_{50} acute detrimental effects on various organs and tissues still need to be measured. This is particularly important having in mind the small size of nanoparticles and their ability to pass through biological barriers (i.e. to get resorbed in oral cavity, and enter the blood stream). Neurotoxicity, hepatotoxicity and nephrotoxicity often do not have to be dose-dependent, or even time-dependent. Therefore, a careful histopatological evaluation, as well as a wide range of biochemical,genetic and physiological tests will need to be conducted to make a definite conclusion and recommendation for future dental application.

Apart from acute, possible chronic toxicity of nanomaterials in dental practice, also remains cause for concern. In industrial workers, as well as scientific, healthcare and other professionals who are due to the nature of their work, chronically come to con-

tact with nanomaterials, some diseases may have higher incidence compared to the general population. Certain respiratory, cardiovascular, urinary and neurological illnesses have been associated to chronic nanoparticle exposure, although the exact causal relationship is not clear.

Some nanomaterials with potential for application in dentistry, are unfortunately thought to have mutagenic and cancerogenic effects, at least in certain concentrations. DNA mutation can occur as a result of numerous biochemical mechanisms. For example some nanoparticles, through oxidative stress, may induce formation of molecules of reactive oxygen species such as hydroxyl radical (-OH), hydrogen peroxide (H_2O_2), and superoxide radical (O_2-). These and other molecules may cause DNA damage and subsequent development of various precanceroses and cancers [15].

In recent years our research group has been focused on potential nanomaterial-induced changes in nuclear structure in oral buccal epithelial cells [34]. For quantification of cell structure, we have applied fractal and textural methods, which are two novel mathematical algorithms commonly applied in biology and medicine for image analysis. In various studies, these methods were used for assessment of changes in chromatin organization during physiological and pathological changes such as programmed cell death, aging etc [35-39]. Fractal and textural analysis could also be used in detection and evaluation of structural changes in the entire buccal mucosa as the which are the result of long-term effects of various substances (i.e. drugs, medicaments, toxins). At present, we are focused on application of these techniques in evaluation of buccal mucose in patients with substance dependence. Preliminary results indicate that fractal dimension (with the values between 1 and 2) and some textural features can have relatively good value in evaluation of altered oral tissues of heroin addicts. It is possible that in the future, these two methods may become an important addition to the conventional, gold standard techniques for evaluation of possible oral toxicity caused by nanomaterials.

5. CONCLUDING REMARKS

Nanotechnology is a novel, rapidly developing discipline that has created numerous opportunities for advancement of current stomatological diagnostic and therapeutic practices. Inorganic as well as organic nanoparticles have unique biological properties that are potentially valuable in virtually all fundamental and clinical medical sciences. With the adequate functionalization, nanoparticles can be successfully applied for creating novel experimental models and assays that may contribute to our current understanding of pathological basis for many oral diseases.

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REFERENCES

[1] E.A. Abou Neel, L. Bozec, R.A. Perez, H.W. Kim and J.C. Knowles // *Int J Nanomedicine* **10** (2015) 6371.
[2] I. Abiodun-Solanke, D. Ajayi and A. Arigbede // *Ann Med Health Sci Res* **4** (2014) S171.
[3] A. Bhardwaj, A. Bhardwaj, A. Misuriya, S. Maroli, S. Manjula and A.K. Singh // *J Int Oral Health* **6** (2014) 121.
[4] L. Yi, Y. Huang, T. Wu and J. Wu // *Neural Regen Res* **8** (2013) 3036.
[5] W. Zhang, Y. Tang, D. Du, J. Smith, C. Timchalk, D. Liu and Y. Lin // *Talanta* **114** (2013) 261.
[6] I. Mejare, S. Axelsson, G. Dahlen, I. Espelid, A. Norlund, S. Tranaeus and S. Twetman // *Acta Odontol Scand* **72** (2014) 81.
[7] P. Kalesinskas, T. Kacergius, A. Ambrozaitis, V. Peciuliene and D. Ericson // *Stomatologija* **16** (2014) 44.
[8] R. Chalas, I. Wojcik-Checinska, M.J. Wozniak, J. Grzonka, W. Swieszkowski and K.J. Kurzydlowski // *Postepy Hig Med Dosw (Online)* **69** (2015) 1140.
[9] S.K. Bhavikatti, S. Bhardwaj and M.L. Prabhuji // *Gen Dent* **62** (2014) 72.
[10] S. Lakshmi and D. Balasubramanian // *International Journal of Dental Science and Research* **1** (2013) 40.
[11] F.G. de Carvalho, B.R. Vieira, R.L. Santos, H.L. Carlo, P.Q. Lopes and B.A. de Lima // *Pediatr Dent* **36** (2014) 85.
[12] S.B. Huang, S.S. Gao and H.Y. Yu // *Biomed Mater* **4** (2009) 034104.
[13] A. Mielczarek and J. Michalik // *Am J Dent* **27** (2014) 287.

[14] P. Tschoppe, D.L. Zandim, P. Martus and A.M. Kielbassa // *J Dent* **39** (2011) 430.
[15] I. Pantic // *Rev Adv Mater Sci* **37** (2014) 15.
[16] R. Foldbjerg, D.A. Dang and H. Autrup // *Arch Toxicol* **85** (2011) 743.
[17] M. Ghosh, M. J. S. Sinha, A. Chakraborty, S.K. Mallick, M. Bandyopadhyay and A. Mukherjee // *Mutat Res* **749** (2012) 60.
[18] K.J. Cross, N.L. Huq and E.C. Reynolds // *Curr Pharm Des* **13** (2007) 793.
[19] R.K. Rose // *Caries Res* **34** (2000) 427.
[20] W. Samprasit, R. Kaomongkolgit, M. Sukma, T. Rojanarata, T. Ngawhirunpat and P. Opanasopit // *Carbohydr Polym* **117** (2015) 933.
[21] S. Kulshrestha, S. Khan, R. Meena, B.R. Singh and A.U. Khan // *Biofouling* **30** (2014) 1281.
[22] A. Oyefusi, O. Olanipekun, G.M. Neelgund, D. Peterson, J.M. Stone, E. Williams, L. Carson, G. Regisford and A. Oki // *Spectrochim Acta A Mol Biomol Spectrosc* **132** (2014) 410.
[23] N. Sasani, J. Vahdati Khaki and S. Mojtaba Zebarjad // *J Mech Behav Biomed Mater* **37** (2014) 125.
[24] W. Wang, F. Watari, M. Omori, S. Liao, Y. Zhu, A. Yokoyama, M. Uo, H. Kimura and A. Ohkubo // *J Biomed Mater Res B Appl Biomater* **82** (2007) 223.
[25] N.J. Wood, H.F. Jenkinson, S.A. Davis, S. Mann, D.J. O'Sullivan and M.E. Barbour // *J Mater Sci Mater Med* **26** (2015) 201.
[26] S. Kim // *Clin. Oral Impl*. **25 (Suppl. 10)** (2014) 170.
[27] H. Huang, Y. Chang, M. Lai, C. Lin, C. Lai and T. Shieh // *Surface & Coatings Technology* **205** (2010) 1636.
[28] R. Dziak, K. Mohan, B. Almaghrabi and Y. Park, In: *Nanobiomaterials in Clinical Dentistry* (Elsevier Inc. New York, 2013).
[29] Z. Shi, X. Huang, Y. Cai, R. Tang and D. Yang // *Acta Biomater* **5** (2009) 338.
[30] T.J. Webster, C. Ergun, R.H. Doremus, R.W. Siegel and R. Bizios // *Biomaterials* **21** (2000) 1803.
[31] V.P. Singh, D.G. Nayak, A.S. Uppoor and D. Shah // *Dent Res J (Isfahan)* **9** (2012) 60.
[32] L. Chen, Q. Yu, Y. Wang and H. Li // *Dent Mater* **27** (2011) 1187.
[33] M. Yasaei, A. Zamanian, F. Moztarzadeh, M. Ghaffari and M. Mozafari // *Biotechnol Appl Biochem* **60** (2013) 502.
[34] I. Pantic, J. Paunovic, M. Perovic, C. Cattani, S. Pantic, S. Suzic, D. Nesic and G. Basta-Jovanovic // *J Microsc* **252** (2013) 286.
[35] I. Pantic, G. Basta-Jovanovic, V. Starcevic, J. Paunovic, S. Suzic, Z. Kojic and S. Pantic // *Nephrology (Carlton)* **18** (2013) 117.
[36] I. Pantic, L. Harhaji-Trajkovic, A. Pantovic, N.T. Milosevic and V. Trajkovic // *J Theor Biol* **303** (2012) 87.
[37] I. Pantic, S. Pantic and G. Basta-Jovanovic // *Microsc Microanal* **18** (2012) 470.
[38] I. Pantic, S. Pantic and J. Paunovic // *Microsc Microanal* **18** (2012) 1054.
[39] I. Pantic, S. Pantic, J. Paunovic and M. Perovic // *An Acad Bras Cienc* **85** (2013) 1063.