Development of an Effective Antibacterial Modification for the Novel Injectable Composite Based on Hydroxyapatite and Cross-linked Gelatin with Potential for Application as an Adhesive for Implants Fixation

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Statement of Purpose: Severe bone injuries due to complicated fractures or critical bone defects followed by tumor resection may require the use of implants. Despite numerous research works on novel implants, there is still no effective solution for their fixation in bones [1]. Hence, our team has started research on the development of a novel bone glue for this purpose. Moreover, invasive operations of implantation are always associated with the risk of hospital-acquired infection, and also biomaterials themselves contribute to the introduction of bacteria into the body. Therefore, effective antibacterial protection is expected from modern biomaterials, and doping with antibacterial agents, such as antibiotics or noble nanometals, seems to be the standard solution [2]. The main aim of our study was to develop and characterize the bioactive modification for injectable composite doped with gentamycin or nanosilver. The impact of these modifiers and the effects of their contents on various properties of the material were assessed.

Methods: Gelatin (Merck, Germany) was dissolved in demineralized water (44% wt/v), hydroxyapatite (88% wt/v; <200 μm article size, Merck, Germany) previously thoroughly mixed with modifiers (0.15, 0.5 or 1.0% wt of powder components; gentamycin sulfate, GMC, Serva, Germany; silver nanoparticles, nAg, ~30 nm, Hongwu International Group Ltd., China) was added to the solution constantly stirred in an ultrasonic bath at a temperature of ~50°C and loaded into a syringe. Next transglutaminase BDF PROBIND TXo (1.8% wt/w of paste; BDF Natural Ingredients S.L., Spain) dissolved in 1 mL demineralized water was poured into a syringe and vigorously mixed. Subsequently, the obtained paste was subjected to injectability tests or pressed into special molds and allowed to cure under ambient conditions. The following research was performed: 1) biostability – evaluation of the percentage of degradation and solubility in PBS solution by UV-VIS spectroscopy (Evolution 220, Thermo Fisher Scientific, USA); 2) injectability – measurement of the max. application time of the paste by injection until its hardening with automatic injector; 3) surface wettability with an optical tensiometer (Attention Theta Life, Biolin Scientific, Finland); 4) microstructure by SEM microscopy (Quanta FEG 250, FEI Company, USA); 5) chemical structure by FTIR spectroscopy (PerkinElmer Frontier, USA); 6) mechanical properties using the Oliver-Pharr indentation method (NanoTest Vantage, MicroMaterials, UK); 7) cytocompatibility – assessment of human osteoblast viability (ATTC-CRL-11372) by MTT and LDH assay and 8) antibacterial effectiveness – bacterial growth inhibition in solution according to the McFarlands standards and a Kirby-Bauer zone of inhibition test with *Staphylococcus aureus* (ATCC 25923 and hospital strain).

Results: The novel composites with antibacterial protection were successfully obtained. All tested modifiers did not significantly affect microstructure, as SEM examinations shown, and did not change the chemical structure as all FTIR spectra were comparable. In addition to the specific peaks for gelatin and hydroxyapatite, another peak was found attributed to the formation of a new Ca-COO^- bond (~1345 cm^{-1}) [3], which confirmed the proper mixing of both components. All specimens were hydrophilic, showing wettability ~73.1±6.9°. The addition of GMC did not significantly affect biostability and injectability. However, the use of nAg negatively affected both properties. The percentage of degradation for specimens with nAg was three times higher than for the control (approx. 20-25%) followed by the increasing injection time (by ~2-3 min). UV-VIS analysis confirmed that, for composites with nAg the solubility of gelatin drastically increased. The mechanical properties of specimens did not change significantly after modifications, Young's modulus being of ~10.51±1.37 GPa, and hardness of ~0.262±0.037 GPa. The obtained composites did not disclose a toxic effect on cytocompatibility (~100-120% of unmodified composite), however, the use of the highest concentration of nAg decreased the viability of the cells by ~15%. The results of LDH release were relatively consistent, the specimens with 0.5% nAg caused an increase in LDH in medium (about ~40%), and those with 1.0% nAg probably completely inhibited the activity of this enzyme. Both GMC and nAg allowed to obtain antibacterial protection, and the increase in their concentrations contributed to the improvement of antibacterial abilities. In the case of a reference strain, specimens with GMC had larger zones on the disk and better inhibited the multiplication, but for hospital strains, nAg showed greater effectiveness. Depending on the medical purpose, it may be possible to use both proposed modifications, however, the reduction of nAg concentration seems to be significant due to the potential cytotoxic effect.

References:

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