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EXTRA-CELLULAR MATRICES FOR TITANIUM IMPLANTS

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Introduction and methods

The success of titanium implants for bone contact is dependent on integration with the surrounding bone tissue, which demands suitable implant surface modification. One approach is the biomimicry of the natural bone extra-cellular matrix (ECM) by coating the implant surface with collagen fibrils, which present binding sites for integrins and are not desorbed when placed in solution. Collagen fibrils can serve as a ground matrix, enabling the inclusion of glycosaminoglycans (GAGs) and proteglycans (PGs) which naturally occur in bone, such as Chondroitin Sulphate (CS), which has been shown to stimulate cell bioactivity in vivo [1], and Decorin. CS and Decorin may be immobilised in collagen fibrils during fibril formation in an appropriate phosphate buffer solution. Synergic effects with growth factors, which can retain their biological activity on collagen, are also possible [2] [3] [4].

In middle ear surgery, adequate mechanical fixation of titanium prostheses to the stapes footplate has not been achieved. Loading an implant whose surface has previously been coated with GAG/PG-containing collagen fibrils with osseoinductive growth factors may result in fixation through osseointegration. The aims of this work are the characterisation of CS- and Decorin-containing fibrils of the collagen types I, II and III for use as coatings, as well as the reaction of primary osteoblasts from human stapes on titanium surfaces coated with these fibrils, both crosslinked and non-crosslinked, in order to select the most suitable coating for animal experiments. The amount of immobilised CS and Decorin in dependence of the ionic strength of the fibrillogenesis buffer was quantified. CS and Decorin were biotinilated and their bioavailability and desorption studied by ELISA. Primary human osteoblasts were obtained from donated human stapes in tissue culture, expanded and plated onto titanium surfaces coated with collagen fibrils. Cellular reactions were investigated with respect to proliferation, alkaline phosphatase (ALP) activity and expression of osteoblastic markers.

Results and discussion

CS/Decorin assays [5], [6] demonstrated the dependence of amount bound per mass unit of fibrils on collagen type and the ionic strength of the fibrillogenesis buffer. Collagen II bound significantly more CS and Decorin than Collagen I and III at both high and low ionic strengths. At high ionic strength a limit for the incorporation of Decorin in Collagen I was reached. Detection of desorbed biotinilated CS and Decorin in phosphate-buffered saline by ELISA showed differences in the desorption profiles of CS and Decorin. Direct ELISA on surfaces coated with fibrils containing biotinilated CS and Decorin showed that both are bioavailable for interactions at the surface.
Osteoblasts from human stapes footplates showed slight differences in proliferation, ALP-activity and expression of osteoblastic markers, depending on the presence of CS or Decorin, the collagen type, and whether crosslinking had been performed. The choice of collagen type appears to have more influence than the presence of Decorin.

Summary

At low buffer ionic strength a higher amount of Decorin and CS is immobilised in fibrils of collagen I, II and III. Collagen type II can bind more CS and Decorin than types I and III. Desorption profiles of CS and Decorin are different. This may affect the release kinetics of growth factors. Primary stapes footplate osteoblast behaviour appears to be influenced by collagen type, crosslinking and presence of CS or Decorin.

Acknowledgements

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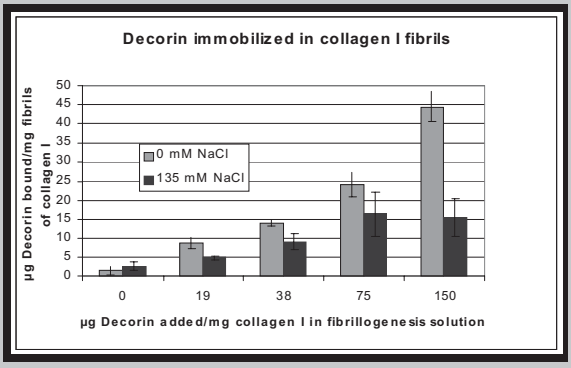
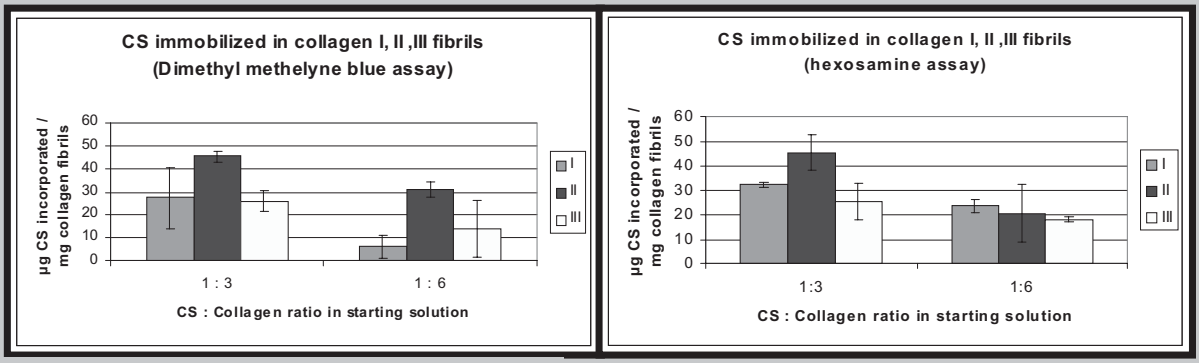


Diagram: Decorin-binding ability of collagen type I fibrils at low (blue) and high (red) buffer ionic strengths. Increase in ionic strength is achieved by addition of 135 mM NaCl to buffer.



Diagrams: CS-binding ability of fibrils of collagen types I (purple), II (red) and III (yellow) measured by Dimethyl methelyne blue assay (left), and hexosamine assay (right).

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METHOD OF MODEL FORMATION OF TRAUMATIC OSTEOMYELITIS OF MANDIBLE IN EXPERIMENT

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Last years cranio-maxillofacial injuries became more frequent, so the quantity of traumatic fractures of mandible is increased and is changed from 67,4% to 85% in dependence of country region, social status of the population. Regardless of the wide application of the modern treatment of the mentioned above pathology, the level of inflammatory complications stay high at the range from 2% to 12%. Last time the character of inflammatory processes has changed. Hard forms of purulent infections on different anatomic areas are frequently met. Traumatic osteomyelitis treatment is a complex problem which demands great attention to it and is to be permanently improved. Adoption of modern methods of treatment and rehabilitation in clinic is to be studied in advance. According to the special literature resources there is no common opinion what animal is to be chosen for experimental studying. In majority of cases rabbits are subjected for experiments. But this model is not quite satisfactory because it is possible to re-create the line of fracture on that area of mandible where the teeth are missing.
Taking into consideration that fact in complex with properties of immunological processes for this kind of animals we have found bone tissue regeneration of all experimental animals during 2 - 3 weeks. That is why last years they have taken a dog as a model for bone tissue regeneration experiment after traumatic fractures and while complications [1, 3, 5, 6, 7, 8, 9]. There is no doubt that immunological status of a dog is different from human one. But denture description, mandible structure, local factors of the oral cavity allow to approximate the experimental model conditions to the clinic situation at the most.

Aim of the research

is to create experimental model of mandible traumatic osteomyelitis when a dog is used as a laboratory animal.

Materials and methods

Experiment was performed on 22 watchdogs. All animals were at the age from 2 to 4 years old and there weight was 9 -10 kgs. Operation has been done under intravenous anesthesia with Sol. Thiopentali-Natrium 10%, 40-45 mg per 1 kg of the animal weight. Using of that anesthesia treatment has permitted to make operations on mandible within 1,5 - 2 hours without additional anesthesia. They have inserted approximately 15 ml of Sol. Thiopentali-Natrium 10% while one operation procedure. That method gives to avoid complications during operation procedures as well as after it.
Operations have been performed in aseptic conditions. Incision has been made parallel in 1 sm to the edge of the mandible. Skeen was cut till the bone. After the periosteotomy and skeletization of horizontal part of mandible made by stomatological equipment, osteotomy has been performed under the angle of 80° - 90° in the region of 35 or 36 teeth. Nerves and capillaries have been cut and mucous membrane of alveolar appendix has been damaged. Fangs in the line of fracture have not been extracted. After that osteosynthesis by dynamic compression plating has been done. The wound was cultivated with 5 ml of Sol. Lincomicini 30%. Layer by layer, they have put stitches in a wound by superamide. Stitches were cultivated by Sol. Iodi Spirituosae 5%. Postoperatively animals had mechanically hard diet (habitual one for this kind of animals).
We successed to take traumatic osteomyelitis in 100% of cases of examined animals experimentally with model clinical descriptions: presence of sinus tract with suppuration, granulation tissue, mobility of mandible parts, presence of big dimensions sequestration with granulation tissue between tissue fragments.

That method of model formation of traumatic ostemyolitis of mandible in experiment is different from known models and has the following advantages:
1) It is easy for application. There is no necessity for additional medical equipment and medicines use.
2) It allows creating the model in short terms.
Also, when formatting the model the same etiological agents of complications for traumatic fractures as for clinical conditions were taken into account. Namely:
1. Damage of bone integrity
2. Factor of bone wound infection
3. Trophism and blood flow stopping in the region of the line of fracture
4. Presence of fangs in the line of fracture