УСТНЫЕ И ПОСТЕРНЫЕ ДОКЛАДЫ

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ISOLATION AND CULTURE OF BOVINE EARLY FOLLICLES GROWN IN COLLAGEN MATRIX GEL

One of the new technologies attracting the attention of reproductive technologists in recent times is the isolation and the culture of preantral ovarian follicles from ovarian tissue for using them as an alternate source of fertilizable oocytes to produce embryos. Well known that the mammalian ovary contains a huge stock of resting follicles. A very small number of these oocytes grow to the final size, mature, and are ovulated. The aim of study was to establish a culture system to support the growth of small bovine oocytes as enclosed in granulosa cell complexes that extend in a three-dimensional collagen matrix supports a spherical structure of follicles and to determine the optimal conditions for in vitro growth and fertilization of early antral animal follicles. Such systems have been established for mouse oocytes but are not applicable to larger animals because it is difficult to maintain an appropriate association between the oocytes and companion somatic cells. The objectives of the study were to investigate the relationship between the morphological statuses of collagen embedded early antral follicles and conditions of culture of the oocytes. In the present study, we compared five culture conditions for growing bovine oocytes and examined the effect of hypoxanthine and hormone on oocytes growth. The oocyte-cumulus-granulosa cell complexes were embedded in collagen gels and cultured for 7 days in 4 different culture systems. When hypoxanthine (4 mM) and FSH (0.02 μg/ml) were added to the culture medium, the number of granulosa cell-enclosed oocytes increased significantly. As result more oocytes enclosed by a complete cell layer and form follicle like structures were recovered from the medium. The percentage of follicles like strictures in this case was 82.9%. After a subsequent maturation culture of the oocytes, 84.2% underwent germinal vesicle breakdown and 14.3% of oocytes were fertilized. The viability of the oocytes to day 8 of culture was 73.1%. The results of in vitro growth of early bovine oocytes in a three-dimension structure demonstrate that using of combination of hypoxanthine with FSH in cultural media can maintained in the complex that developed follicle like structure similar to that observed in ovary. The culture system has the potential to form the basis of oocytes in vitro growth system for the production of mature oocytes and the defined nature of the system makes it suitable as a tool for investigating early oocytes development. Finally, the culture of intact follicles within ovarian stromal tissue provides a unique opportunity to examine the regulation of cell differentiation and follicle growth, particularly at preantral stages. Our experiments suggest that it may be more difficult to maintain the proper association between the oocytes and granulosa cells on a collagen substrate in large animal species and the conditions of growth and fertilization in vitro should be improved and requires addition in-depth study.

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MIGRATION ASSAY OF HUMAN GINGIVAL FIBROBLASTS ON A BIODEGRADABLE MAGNESIUM SURFACE

INTRODUCTION: Since several years there is a high interest in magnesium as biodegradable metal implants for dentistry due to their excellent biocompatibility and their low elastic moduli, similar to natural bone, which prevent stress shielding. The integration of gingival tissue at the implant abutment plays a crucial role for implant success. The aim of this study was to investigate the migration of human gingival fibroblasts (HGF) on magnesium. Therefore, a migration assay adjusted to the specific requirements of magnesium has be to be developed.

METHODS: Primary HGF, labelled with fluorescent dye Cell-Tracker Red were cultivated for 24 h. Cells were seeded in 24-well-plate on plastic as control and pre-corroded magnesium foils by using a silicone insert and were allowed to attach for 24 h to form a confluent monolayer. Before seeding, each slide was "pre-corroded" in 10 mL culture medium for 3 days at 37°C without CO_2 exchange. After scratching and washing the monolayer, the slides were placed with the attached cell-site down. Imaging was started using an inverted microscope with a live cell imaging system over a period of 50 hours.

RESULTS & DISCUSSION: This study shows the first migration assay developed for biodegradable magnesium. Pre-corrosion of the magnesium led to an overall increased cell adhesion and facilitated the migration assay. The migration of HGF on magnesium is about 1.7x times slower than on tissue culture plastik, but even fast than on titanium-zirconium surfaces of similiar roughness. This assay has now been established as a tool to optimize biodegradable magnesium surfaces.

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STANDARDIZED DEFECT SHEEP MODELS AS A PREREQUISITE FOR OVINE NCSCS BONE REGENERATION RESEARCH

The use of animal models is an essential step in the testing of new biomaterials prior to use in humans. There are many animal models for bone regeneration

research, each having differences in bone remodelling and bony architecture, with potential advantages and disadvantages. Sheep have been used as an animal model for various fields of biomedical research. Mandibular defect models and extraoral models in sheep, including tibia, lower femur, and maxillary sinus have been investigated. These models allow us to compare osseous healing in different bone types (trabecular cancellous bone or dense cortical bone). Bone tissue engineering using ovineNCSCs offers a promising strategy for healing severe bone injuries by utilizing the body's natural biological response to tissue damage in conjunction with engineering principles. Therefore, we decided to test the potential of this biological strategy for tissue-healing enhancement in a big animal model, the standardized defect sheep model, in order to have clear indications on the most suitable augmentation approach for a future clinical application.

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VITROCERAMIC COATINGS OBTAINED BY MEANS OF LASER ABLATION, WITH APPLICATIONS IN DENTISTRY AND ORTHOPEDICS

In dentistry and orthopedics, metals and alloys are the most used inert materials for bone substitution because of their excellent mechanical properties. However, there is the possibility of adverse reactions due to surface corrosion in the physiological environment. Thus, to make metallic implants more biocompatible, they are coated with layers of different natures: polymeric, glassy, ceramic etc. In this context, exploring new bioactive vitroceramic coatings containing both a glassy matrix and a distribution of crystals seems to be an appropriate solution for performance improvement. The synthesis of the mentioned materials was performed by a physical deposition method, namely laser ablation. After setting the oxide compositions, the processing parameters were optimized in order to attain high quality vitroceramic films on top of Ti or Ti-Zr substrates. By employing a vitreous matrix with amorphous structure, the material is prone to cover itself with a thin layer of silica gel after dissolution in the physiological environment, which favors the deposition of apatitic phases. On another hand, the presence of embedded crystalline phases may lead to an increased biocompatibility. The material properties were investigated by X-ray diffraction, electron microscopy and associated techniques, FT-IR and Raman spectroscopy, as well as biological tests, providing information on structure, morphology, biocompatibility and bioactivity.

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A RADICAL SWITCH IN CLONALITY REVEALS THE FORMATION OF A STEM CELL NICHE IN THE EPIPHYSEAL GROWTH PLATE

The majority of disorders affecting final height converge on thin cartilage discs, called growth plates or epiphyseal plates, which are located near the ends of all growing long bones. Growth plates provide a continuous supply of cells that are crucial for the maintenance of normal bone growth. However, how these discs maintain themselves is not known. The generally accepted view is that chondro-progenitors within the growth plate fulfill this function and the consumption of these progenitors leads to the fusion of the growth plate and the cessation of growth. However, this has never been functionally proven. Employing clonal genetic tracing, we show here that in mice longitudinal growth during the fetal and neonatal period occurs via small clones arranged into multi-clonal columns. Such a clonality pattern strongly supports the idea of direct depletion of the progenitors during fetal and neonatal bone growth. In contrast, later in life the clonal pattern drastically changes, with mono-clonal chondrocyte columns formed and a 2-5 fold increase in clone size. This radical switch in clonality suggests that chondro-progenitors acquire a capacity to renew themselves, as no other drastic changes in cell kinetics can be observed. Interestingly, this self-renewal behavior coincides with the formation of the secondary ossification center, the expression of stem cell markers and of symmetric cell divisions. Furthermore, we show that these self-renewing progenitors can be expanded by specifically activating the mTORC1-signaling pathway in the growth plate, suggesting a novel target for the development of new treatments of growth disorders. Thus our data suggest that a stem cell niche is formed postnatally in the epiphyseal growth plate and that this niche is essential for the maintenance of postnatal bone growth.

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VITROCERAMIC THIN FILM COATINGS DEPOSITED ON METALLIC SUBSTRATES BASED ON TI BY LASER ABLATION

Pulsed laser deposition (PLD) method is used to deposit bioceramic thin film coatings on metallic substrates. In our research, we used this method to deposit abiovitroceramic thin film coating on titanium or Ti-Zr alloy substrates. The composition of the oxidic targets was selected from SiO_2—CaO—P_2O_5—(CaF_2) systems and the corresponding masses were prepared using the sol—gel method and then pressed and thermal treated at 1300°C for 2 h, in oxidative atmosphere. The depositions by PLD method were performed in oxygen-rich atmosphere (100 mTorr) and the metallic substrates were heated at 400°C. The films were analysed by different experimental techniques: X-ray diffraction, electron microscopy (scanning — SEM, EDX and transmission — HRTEM, SAED) and infrared spec-