VERIFICATION OF BIOLOGICAL PROPERTIES OF POLY-ε-CAPROLACTON (PCL) AS THE MATERIAL DEDICATED TO MEDICINE AND BIOTECHNOLOGY

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Abstract

The aim of the study was evaluation of possibility of poly-ε-caprolacton (PCL) application as potential material for production of medical devices, as catheters for obtaining and transporting of embryos as well as dishes for embryos culture in vitro and covers for cryoconservation. The possible application of this biomaterial needs verification of its biological properties on embryos culture. The foil discs made of policaprolacton, thickness 0.5 mm, diameter 3.5 mm, were prepared in two forms: the baseline one (nPCL) and thermally modified by freezing with liquid nitrogen (mPCL). The verification of PCL bioconcordance was performed by evaluation of 102 pig embryos. To evaluate poly-ε-caprolacton bioconcordance we performed 5-day long culture of embryos on the evaluated material, not frozen (nPCL) and frozen in liquid nitrogen (mPCL) and additionally culture after short contact with poly-ε-caprolacton, lasting 15 minutes (nPCL-15). In all evaluated study groups the development of embryos was suppressed shortly after transfer to the culture with PCL. In the control group. 74%-78% of embryos reached blastocyst stage. Polycaprolacton cannot be used as the material for catheter production used in biotechnology of animal reproduction and other materials used for in vitro culture and cryoconservation.

Keywords: *embryos, poly-ε-caprolacton (PCL), culture, catheter, pigs*

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Introduction

During the last few years, the big progress was made in biotechnology of animals reproduction, concerning i.a. fertilization and obtaining of embryos in vitro (IVF/IVM - in vitro fertilization / in vitro maturation), embryos preservation in low temperature, obtaining of transgenic animals for breeding and medical purposes as well as sexing and cloning of embryos. Development of these biotechnological disciplines is not possible without effective methods of embryos transfer.

The main limitation of embryos transfer is the lack of catheters for embryos obtaining and transferring which would have desirable and confirmed biological properties to ensure high bioconcordance and low toxicity. Catheters used routinely for embryos obtaining and transfer are tools used for insemination, urology and other medical purposes, but their bioconcordance with embryos was never confirmed.

For these reasons, in our study we attempted to develop the modern set of catheters designed for the contact with embryos, based on the biomaterial characterized by good biological and mechanical properties, with optimal resilience and plastic properties, with possibility of free shaping and expanding a straw of the diameter of 1–2 mm. The material that hypothetically fulfills biological requirements and at the same time gives possibility of free shaping is the resorbable polymer used for the long time in many fields of medicine - poly-ε-caprolacton (PCL). Because of its biological and physical properties it was taken into consideration to use this biomaterial for in vitro embryos culture and for cryoconservation. However its potential application requires verification of its biological properties in embryos culture. The positive verification of bioconcordance and low toxicity can allow for application of poly-ε-caprolacton as the material for production of catheters dedicated for biotechnology of animal reproduction and gynecology.

Materials and methods

The material used for the study were discs made of PCL foil of thickness 0.5 mm and diameter 3.5 cm. Experiments were performed with employment of 102 pigs embryos, at the development stage of two to four blastomers. Embryos were obtained surgically by rinsing out from the fallopian tube. The embryos donors were ten female pigs, age six to eight, breeding in one farm. To obtain the highest possible number of embryos at the same time the donors underwent hormonal synchronization of sexual cycle and superovulation according to the schedule presented in the TABLE 1.

TABLE 1. The schedule of oestrus and superovulation synchronization.

Day	Hour	Hormone	Donors
0	8^{00}	PMSG or eCG	1000–1500 j.m.
3 (72 hours after PMSG)	8^{00}	hCG	750–1000 j.m.
4 (24 hours after hCG)	8^{00}		Insemination
	20^{00}		
5 - 11	8^{00}		Embryos collection
PMSG-pregnant mare's serum gonadotropin; eCG-equine choriogonadotropin; hCG-human chorionic gonadotropin			

On the third day after surgery animals were sent back to the owner for further breeding. The rinsed embryos were transferred to collective dishes with PBS filled up with 20% fetal bovine serum. The embryos were evaluated under stereoscopic microscope (magnification 100x); after evaluation they were transferred for in vitro culture. The embryos culture lasted five days and was performed in medium NCSU-23, in an incubator, in the temperature 39°C and the atmosphere with 5% of CO_2 in the air. Obtained embryos were divided into five groups: one control group and four study groups: K–control embryos culture (n=38), nPCL-1 –culture of embryos with continuous contact with not frozen

TABLE 2. Effectiveness of pigs embryos culture.

Group	Number of embryos in culture (2–4 blastomers)	Stage of development ofter 5 days culture	
		2-4 blastomers	Blastocyste
I Stage			
Control	9	2	7
nPCL-1	17	17	0
mPCL-1	10	10	0
II Stage			
Control	29	6	21
nPCL-2	20	20	0
nPCL-15"	17	17	0

nPCL-1 – culture of embryos with continuous contact of embryos with not frozen PCL; mPCL-1 – culture of embryos with continuous contact of embryos with PCL frozen in liqiud nitrogen; nPCL-2 - culture of embryos with continuous contact of embryos with purified PCL; nPCL-2-15" – culture of embryos after short (15 minutes) contact with purifeid PCL

PCL (n=17), nPCL-2–culture of embryos with continuous contact with PCL frozen in liquid nitrogen (n=10), nPCL-2 – culture of embryos with continuous contact with purified PCL (n=20) and nPCL-2-15" - culture of embryos after short (15 minutes) contact with purified PCL (n=17).

The experiment was divided into two stages. In the first, stage, embryos were cultured for five days on not frozen polycaprolacton (Group nPCL-1) and PCL frozen in liquid nitrogen (Gourp mPCL-1). For the second stage, the new production of policaprolacton was performed, with PCL dried for 24 hours in the temperature of 40^0C. The aim of the prolonged and strictly controlled drying was to exclude the potential possibility of contamination of PCL with dissolvent. In the second stage, we gave up the embryos culture on the frozen material. Culture was performed on the not frozen, purified polycaprolacton only (Group nPCL-2). We additionally add one group in which the contact of embryos with dedicated material was limited to 15 minutes (nPCL-2-15").

Results and discussion

The performed experiment was the first attempt of verification of biological properties of the biomaterial for the dedicated application in biotechnology of reproduction to the best of authors' knowledge This kind of experiment which aimed to confirm the properties of biomaterials in the contact with embryos were not performed so far and they are no data concerning this subject in literature. In the performed study the possible wide employment of polycaprolacton was taken into consideration, mainly as the potential material for the production of catheters for embryos transfer, but also dishes for in vitro culture as well as straws and probes for cryoconservation. In the case of application such catheters as the tools for transferring of embryos in vitro and in vivo, the most important feature of biomaterial, because of its application, would be bioconcordance and facility of processing of polymer dedicated as the material temporarily introduced into organism. These conditions are fulfilled by bioconcordant polymer poly-ε-caprolacton (PCL). PCL is the material that has been known for over 40 years. During this time, its biological properties were verified many times in different experimental models. Due to its properties it is widely used in medicine, i.a. for the production of surgical sutures that undergo biodegradation or implants for cardiology, ophthalmology, transplantology and other fields of medicine [10-13]. Additionally, easy methods of processing this material, with the possibility of forming it into any shape (i.a. polymer tubes, 10-40 cm long) make PCL the potentially useful material for production of catheters for embryos transfer. Because sensitivity of embryos to toxins is extremely high, much higher then somatic cells, it was necessary to confirm biological properties of poly-ε-caprolacton in in vitro embryos culture.

The experiment was based on the culture of embryos in five days long incubation with chosen material. In the first stage, in two study groups nPCL-1 (0/17) and mPCL-1 (0/10) none of the cultured embryos developed to the blastocyste. In the control group, we obtained blastocyste stage in 78% of embryos (7/9). In both study groups (nPCL-1 (0/17) and mPCL-1 (0/10)) after five day culture all embryos had the same stage of development as at the moment of the transfer to culture. The restriction of embryos development took place short after transferring embryos to culture on polycaprolacton. We took into consideration two possible causes of development restriction during culture: the mistake during production of the polycaprolacton with not proper drying of foil with possibility of leaving the dissolvent or the strong toxic impact of PCL on the embryos. To exclude the possible impact of dissolvent, the PCL was produced again with very careful drying of the foil as described above. Additionally, we established the additional group in which contact of embryos with biomaterial was limited to 15 minutes. In the instituted schedules of embryos transfer it is extremely important to transfer embryos from culture to the natural environment, i.e. Fallopian tube or uterus as fast as possible, depending on the stage of embryos development. As the rule, the contact of embryos with catheters is restricted to 1–2 minutes. For this reason we decided to evaluate toxicity of biomaterial after short (15 minutes) contact of PCL with embryos. The results were similar as in the first stage of the experiment. Blastocystes developed only in control group (74%, 21/29). In two study groups, nPCL-2 and nPCL-15´, no blastocystes were observed. Development of the most of embryos was stopped shortly after transfer to culture. These results were similar as for the first part of our experiment.

Conclusions

Policaprolacton is toxic for embryos in in vitro culture. Because of proved cytotoxic impact on embryos it cannot be used as material for production of catheters used for biotechnology of animals reproduction and other medical devices used for in vitro embryos culture and cryoconservation.

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CORRELATION OF COMPOSITION AND STRUCTURE OF SHARK TEETH

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Abstract

In contrast to mammalian teeth with the biomineral phase hydroxyapatite, the shark teeth contain harder mineral phase fluoroapatite with partial substitutions of phosphate by carbonate and of fluoride by hydroxide [1]. Their excellent mechanical properties are due to a special hierarchical structure of the constituting fluoroapatite crystals and organic matrix [2]. The two main structural elements of teeth, i.e. hard and mineral-rich enameloid on the outside and softer and less mineralized dentin on the inside, were structurally, chemically and mechanically characterized [3]. The teeth of two different shark species mako shark (Isurus oxyrinchus) and tiger shark (Galeocerdo cuvier) were investigated and their hierarchical structure by high-resolution scanning electron microscopy presented (Fig.1). X-ray diffraction showed that the inorganic matrix of both enameloid and dentin consisted of fluoroapatite, with a high crystalline phase in enameloid and nanocrystalline phase in dentin. FTIR-spectra of the shark teeth showed the characteristic bands of biological apatite. It was found by thermogravimetry that dentin had a higher content of water, organic matrix and carbonate than enameloid. To investigate the mechanical properties of the teeth in longitudinal and cross sections, nanoindentation and Vickers microhardness were carried out. Both methods gave comparable results: the enameloid of both shark teeth was approximately six times harder than the dentin with an isotropic hardness (longitudinal or cross section).

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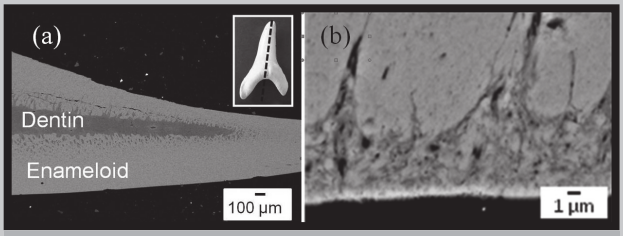

FIG.1. Scanning electron micrographs (back-scattered electron mode) of the axial cross section of a tooth of Isurus oxyrinchus showing the interface between dentin and enameloid (a) and also the topological arrangement of the apatite crystals (b)

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