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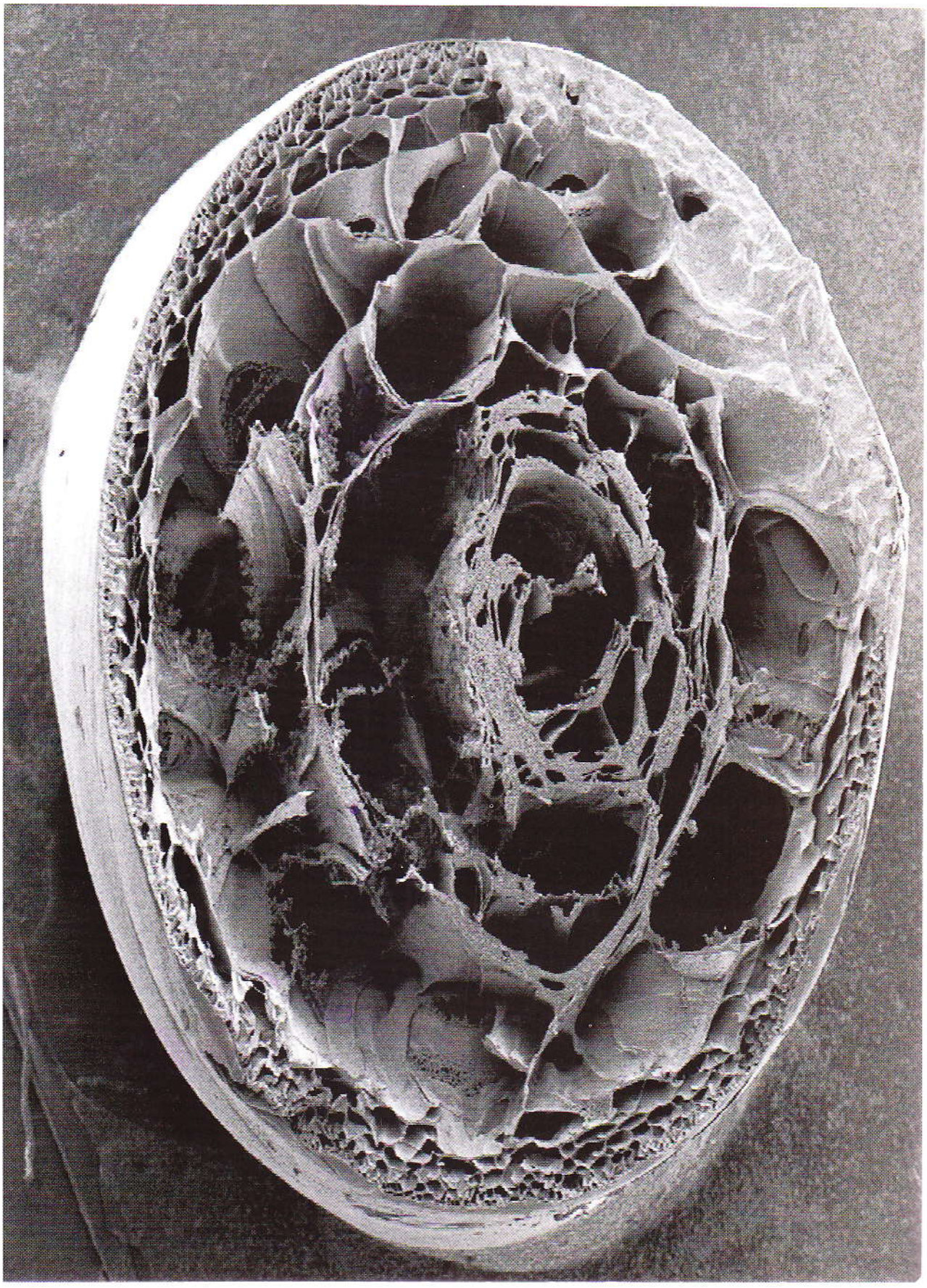

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INŻYNIERIA BIOMATERIAŁÓW

BACTERIAL ADHESION TO IMPLANTABLE MATERIALS

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[Engineering of Biomaterials, 37, (2004), 4-5]

Introduction

Staphylococcus aureus and *S. epidermidis* are human pathogens that colonise implant surfaces. They are of increasing importance due to the rise in antibiotic resistance [1]. *S. aureus* is one of the main causes of metal-biomaterial, bone joint and soft tissue infections [2], and is distinct from *S. epidermidis* which is an opportunistic bacteria, associated with catheters and other indwelling medical devices [3]. *S. aureus* and *S. epidermidis* are both capable of forming biofilms which can be difficult to clinically treat because the bacteria in the interior of the biofilm are protected from phagocytosis and antibiotics [4], hence the need to prevent bacterial adhesion to implants. This may be possible by modifying the topography/chemistry of the implant surface, or coating it with an antimicrobial/protein resistant coating. Here we describe methods for the visualisation and quantification of *S. aureus* and *S. epidermidis* adhering to different implantable biomaterials.

Methods

To visualise S. aureus and S. epidermidis adherence on different surfaces, bacteria were cultured on the various modified biomaterial surfaces in brain heart infusion broth (BHI) at 37°C over several time periods, then fixed with 2.5% glutaraldehyde in buffer, post-stained with 1% osmium tetroxide, dehydrated, critical point dried, coated with Au/Pd, and visualised with a scanning electron microscope using a backscattered electron detector [5]. To quantify the amount of bacterial adhesion, adherent bacteria were stained with fluorescent 5-cyano,2-ditolyl tetrazolium chloride (CTC), and visualised with a Zeiss Epifluorescence microscope fitted with an Axiocam camera [6]. The density of live bacteria on the surfaces in each image was counted using KS400 software. On surfaces that auto-fluoresce, adherent S. aureus and S. epidermidis were detached by sonication in Tween 80, then stained with a live/dead assay (Molecular Probes). The amount of bacteria present were counted using a Partec PAS flow cytometer.

Results

SEM images showed variations in the adhesion of *S. aureus* and *S. epidermidis* to the surfaces (FIG.1). No differences in the amount of S. aureus adhesion were observed on standard titanium (TS), electropolished titanium (TE) and TS coated with nitrogen ions (TIG) surfaces, slightly more were found on TS coated with polymer for promoting cell adhesion (TAST), but few were found on the TS coated with hyaluronate acid (THY) (FIG. 2). In comparison to the uncoated titanium surface (TiS), few bacteria were seen on the PLL-g-PEG (PEG) and PLL-g-PEG functionalised with RGD surfaces (PEG-RGD) (FIG. 2b).

Flow cytometry results quantified the difference in the amount of *S. aureus* and *S. epidermidis* adhering to vari-

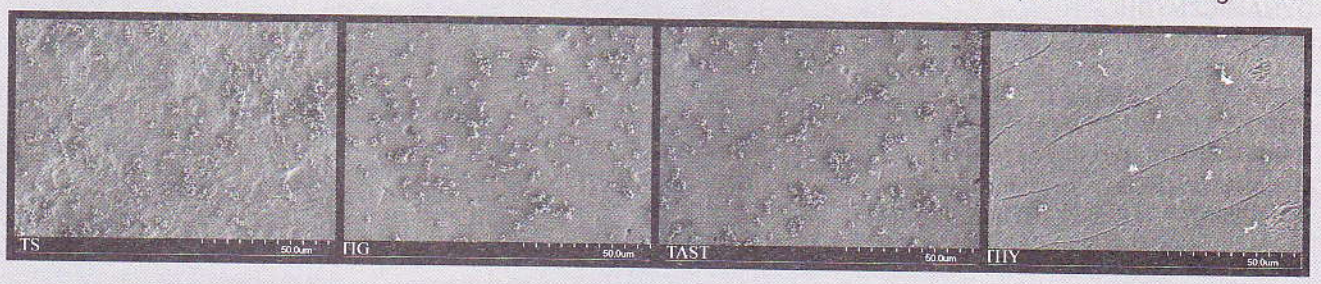

FIG. 1. SEM images of *S. aureus* adhering to different materials.

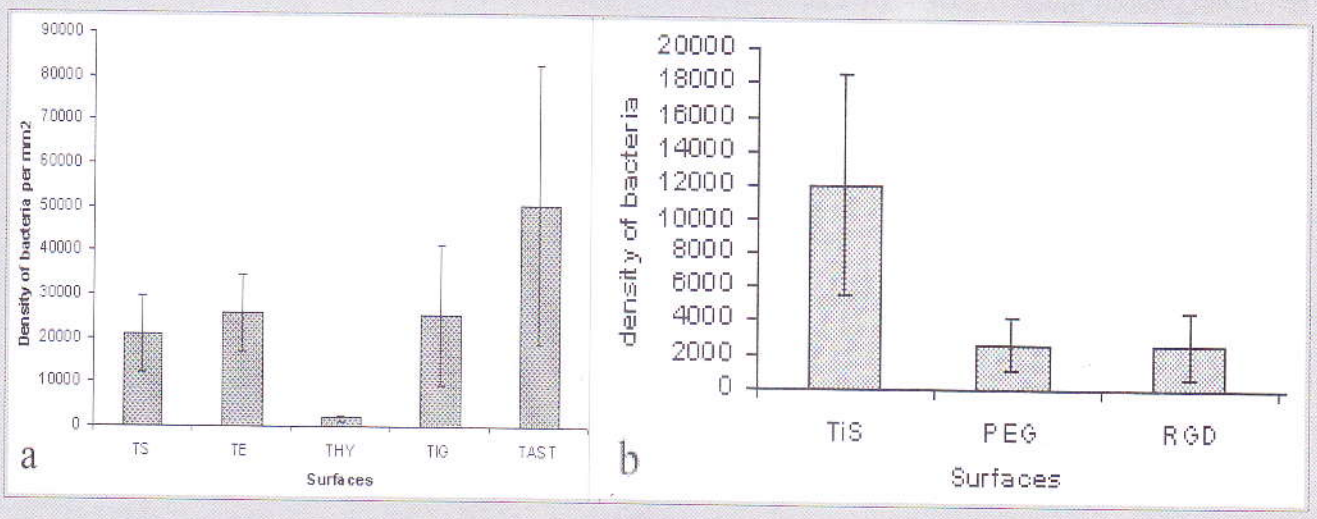

FIG. 2. Graphs showing the density of bacteria on different surfaces after 1h of culturing. a) on TS, TE, TIG, TAST and THY; b) TiS, PEG and PEG-RGD.

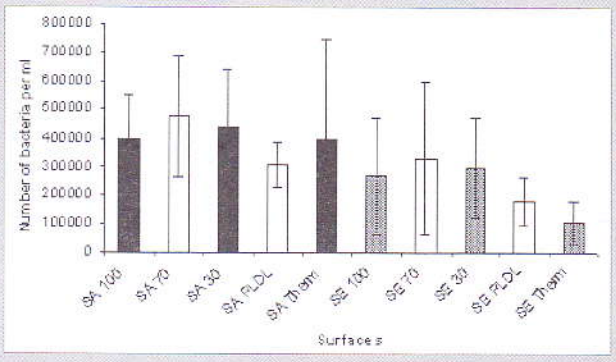

FIG. 3. Graph showing flow cytometry results from SA (*S. aureus*), and SE (*S. epidermidis*) adhering to different polyurethanes.

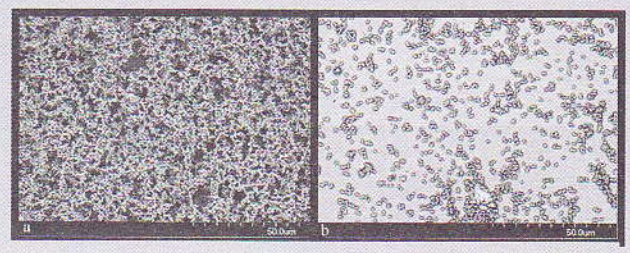

FIG. 4. SEM images of a) *S. aureus* and b) *S. epidermidis* adhering to 70% polyurethane.

ous surfaces. The example shown in FIGURE 3 confirmed SEM observations (FIG.4), that more *S. aureus* adhered to the surfaces than *S. epidermidis*.

Discussion and conclusions

These results show that different methods can be used to study the adhesion of bacteria to biomaterials in vitro. SEM is useful for morphology and general observations, and depending on the material (polymers tend to auto-fluoresce), either fluorescence microscopy or flow cytometry can be used to quantify the amount of adherent bacteria.

Acknowledgements

Thanks to Dr Kati Gorna (ARI, Davos), Dr John Disegi (Synthes USA), Dr Lukas Eschbach (RMS, Bettlach, CH), and Dr Samuele Tosatti (ETH Zurich, CH) for the surfaces.

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THE RADIOIMMUNO-ASSAY OF CORTISOLE LEVEL IN MIXED SALIVA FROM THE PATIENTS WITH MULTIPLE DENTAL CARIES

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[Engineering of Biomaterials, 37, (2004), 5]

Caries is known to be accompanied by the metabolism impairment in a human organism. These changes involve the oral liquid medium, which is mixed saliva. A great number of articles are devoted to the protective role of the oral fluid, its participation in oral metabolism. However little attention is paid to the study of cortisole level in mixed saliva from patients with different degrees of caries.

The aim

of presented article was to study of cortisole level in mixed saliva from patients with different degrees of caries.

Materials and methods

For this purpose we have studied 3 groups of patients from 15 to 25 years of age. The samples of saliva for research were collected in the morning time, before breakfast in disposable sterile tubes.
Before testing, samples were stored in liquid nitrogen at temperature - 196°C. The hormone levelsin oral fluid was determined by using cortisole marked with Iodine 125 (Steron-C-125 I) in the detector of Gamma-camera measuring the speed of sedimentation in each sample in 1minute. After that we determined the cortisole level in nMol/l.

Results

Analysis of the results of our research shows that there are considerable differences in concentration of cortisole in saliva from people with low and high intensity of caries ($p < 0,01$) and low and middle intensity of the process ($p < 0,05$). Differences between middle and high caries intensity groups weren't reliable ($p < 0,05$).

Conclusion

On the basis of our research we can say that change of cortisole level in mixed saliva reflects growth of cariesogenic situation in the oral cavity. Consequently, this test can be used for diagnostic purposes and for determination of the effectiveness of treatment and preventive measures.

CLINICAL APPLICATION OF RESORBABLE POLYMERS IN GUIDED BONE REGENERATION

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[Engineering of Biomaterials, 37, (2004), 6]

Introduction

Long segmental diaphyseal bone loss often results from high energy trauma like blast injury, osteomyelitis or wide excision of malignant conditions. Treatment of this long segmental diaphyseal defects remain a difficult clinical problem. In the literature, many authors have reported that bone loss more than 2.5 cm always require bone grafting. This is probably the critical size defect in human. Non-vascularized bone graft frequently fails if the defect is longer than 6-7 cm. 2.5 cm is probably the critical size defect is human and 7 cm is likely the critical size for non-vascularized bone graft. Various treatment methods are adopted currently to address this problem, including vascularized bone graft, distraction osteogenesis and massive allograft. However, all these methods are associated with a lot of problems.

Successful guided bone regeneration has been achieved in skull bone and jaw bone using resorbable allograft. Bone regeneration in long segmental defect and relatively small defect in tumour excision has been achieved using resorbable polylactide scaffolds.

Methods and materials

10 patients with bone defect of sizes up to 6 cm due to various causes including benign tumour, osteomyelitis & fractures were treated with resorbable polylactide scaffold impregnated with marrow blood which contains stromal cells. In cases with infection, antibiotics was also loaded into the scaffold and in this situation, the scaffold also served as a drug delivery device. The patients have assessed regularly with X rays and clinical symptoms.

Results

Serial X ray evaluation and clinical evaluation revealed presence of bone regeneration. The limbs enjoyed satisfactory function and there was minimal donar site morbidity and major surgery can be avoided.

Discussion

Selected cases are treated with guided bone regeneration which would be treated otherwise by conventional technique. Vascularized bone transfer has limited supply and involves a major operation. There is always a chance of vascular complication and there is donor site morbidity. Distraction osteogenesis has a limitation of length that can be lengthened and requires a prolonged placement of external fixation. There is a high chance of traction injury to nerve and other soft tissues. Massive allograft requires a prolonged period, in terms of decades, for complete creep substitution. There is also a high incidence of disease transmission and infection. Therefore there is a constant demand for bone substitute which can bridge long segmental defect effectively with minimal morbidity and can heal in reasonable time frame. The affected limb can be rehabilitated and bear weight for functional restoration as early as possible. These early results are promising.

INJECTABLE POLYURETHANES FOR THE TREATMENT OF THE OSTEOPOROTIC SKELETON

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[Engineering of Biomaterials, 37, (2004), 6-8]

Introduction

Bone loss resulting from osteoporosis increases the risk of fractures. The "at risk" population is estimated to be 28 million in the US and 100 million worldwide. Among fractures of osteoporotic bones compression fractures of the vertebral body are the most demanding to treat. Common treatment involves injecting of methylmethacrylate (MMA) cement into the vertebral body to restore the height of a collapsed vertebra (vertebroplasty, kyphoplasty).

Both procedures have several drawbacks including leakage of MMA cement outside of the vertebra, soft tissue damage, pressure on nerve roots and/or the spinal cord resulting in pain, paralysis necessitating cement removal, migration of cement to the lungs, which may cause pulmonary embolism, respiratory and cardiac failure, and even death. Increased stiffness of the segments resulting from the presence of injected cement, often leads to fractures of the vertebrae adjacent to those treated. The MMA cements have high rigidity and, in consequence, there is a mismatch in Young's moduli between cement and osteoporotic bone. Tissue necrosis due to high polymerization temperature of MMA and high monomer toxicity can be yet another problem.

A possible solution to these problems could be the use of new injectable polymeric materials that set at lower temperatures, possibly based on nontoxic monomers and having lower moduli than those of poly(methylmethacrylate) (PMMA) cements. The latter property can be achieved, for example, by incorporating an elastic component in the polymer chain or by developing porous structure in the setting cement. In addition, such injectable materials could be loaded with antiresorptive drugs preventing further bone loss and osteogenic drugs promoting new bone formation.

If the osteoporotic bone of the vertebrae possesses potential to regenerate, it might be beneficial to use biodegradable cements, allowing new bone to fill the space formed by degrading cement.

Candidate materials for such injectable cements are seg-

mented polyurethanes, which can be synthesized with a broad range of mechanical and biological properties.

Injectable polyurethane cements can potentially be prepared in two ways. In the first approach the linear polymer is dissolved in an appropriate solvent to obtain solution with viscosity suitable for injection. The second route is similar to that of MMA cements, i.e. two or more monomers are premixed and subsequently injected into the vertebrae where polymerization is completed as a result of catalysis.

This study reports on the process of designing new biocompatible injectable materials for the treatment of the osteoporotic skeleton, based on linear segmented polyurethanes and/or hybrids consisting of these polymers and nanosize calcium phosphate salts.

Experimental

Polymers: Experimental linear polyurethanes with a molecular weight in the range of 70.000 - 110.000 dalton, designed for cancellous bone graft substitutes and for tissue engineering. The polymers were based on aliphatic noncarcinogenic diisocyanates, ε-caprolactone diol, polysaccharide diol and biocompatible catalyst. The hard segment contents of these materials were 60 and 70%, respectively.

Polymer solutions for injection: Injectable polyurethane solutions were prepared by dissolving the polymers in dimethyl sulfoxide (DMSO) and/or methyl-2-pyrrolidone (NMP). Both solvents are allowed by the FDA allowed for contact with tissues (PMP - permited daily exposure = 48.4 mg/day). Nanosize CaP salt was hydroxyapatite (HA) with broad particle-size distribution (Merck, Germany).

Materials characterization: Polymerization kinetics: calorimetry, infrared spectroscopy. Cement setting temperature: calorimetry. Composition: infrared spectroscopy. Absolute HA content: thermogravimetry. Cement structure: SEM. Mechanical properties: compressive strength and Young's moduli at 10% deformation. Water uptake: gravimetric. In vitro degradation: phosphate buffer, 37°C, pH changes. Mass loss upon degradation: gravimetric. Additional tests: extractables, degradation products, cytotoxicity, biocompatibility.

FIG. 1. Nanosize HA crystals in various solvent systems.

FIG. 2. Porous structure developing in PU - nanosize HA hybrids in an aqueous environment.

Solvent systems	Initial concentration of HA (%)		
	2	6	8
DMSO	1.08	4.58	6.44
NMP	1.05	4.50	6.20
EtOH	0.51	2.67	4.10
Water	0.49	2.45	4.40
DMSO/NMP 50/50	0.98	4.57	6.23
DMSO/NMP 20/80	0.86	4.49	6.16
DMSO/NMP 80/20	0.96	4.61	6.20

TABLE I. Stability of HA suspension in various solvents after 24 hr of storage.

Results

For the polymers used in the study the concentrations of solutions suitable for injections were 20 wt-% for the poly-

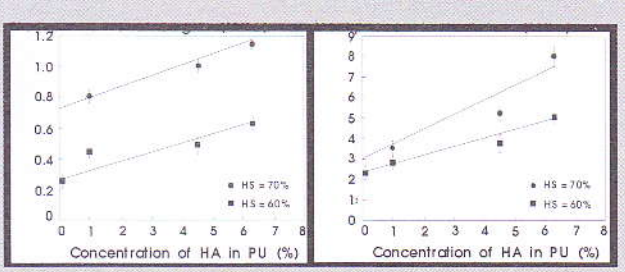

FIG. 3. Mechanical properties of injectable polyurethane materials.

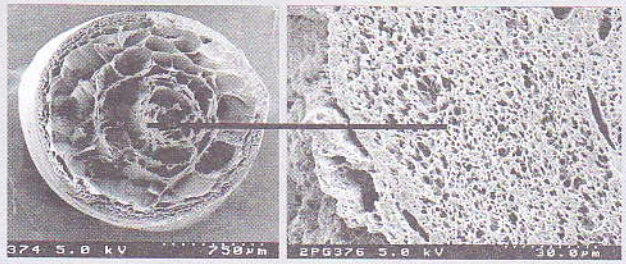

FIG. 4. Porous tubular scaffolds for nerve regeneration.

mer with a hard segment content of 60%, and 16 wt-% for the polymer with a hard segment content of 70%. Stable suspensions of nanosize hydroxyapatite HA could be obtained by using suitable high-viscosity, high-boiling point solvents (TABLE I).

Injectable hybrid materials can be produced by dissolving new biocompatible polyurethanes of varying elasticity in the suspensions of nanosize hydroxyapatite crystals in such solvents. The total amount of HA that can be loaded into the polyurethane solution depends on the stability of the HA suspension, i.e. the quality of the solvent. The hybrid materials solidify in an aqueous environment as a result of solvent replacement by water. Solidification is accompanied by the development of porous structure of varying pore size and geometry (FIG. 2).

Due to highly porous structure, the mechanical properties of the PU materials and the PU - HA hybrids are far from those required for the treatment of osteoporotic bone (FIG. 3).

Mechanical properties can be enhanced by reducing the pore-to-volume ratio in the hybrid, increasing the hard segment content in the polyurethane, and/or increasing the HA load in the hybrid. The injectable materials based on polyurethane solutions and nanosize hydroxyapatite may have limited applications in vertebroplasty due to the amount of solvent required to permit injection of the material. These solutions are, however, excellent systems for the preparation of porous scaffolds for tissue repair and regeneration (FIG.4).

Conclusions

Injectable polyurethane and polyurethane - nanosize calcium phosphate cements for vertebroplasty should be based on systems consisting of two or more monomers that are premixed before injection and solidify in the vertebrae as a result of a catalytic process.

EXPERIMANTAL WAY TO DETERMINE EFFICIENTY OF ACUPUNCTURE AND ANALOGOUS TREATMENT IN ALLOGENIC RHINOPLASTY

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[Engineering of Biomaterials, 37, (2004), 8]

Aesthetic surgery of innate and acquired nose pathology takes 58,90% of the total amount of aesthetic operations made in cranio-maxillofacial area. Grate attention in plastic surgery is pied to the stimulation of reparative and compensatory processes which are turned to the quickest rehabilitation of connective body structures, as well as in rhinoplasty.

Aim

of this abstract deals with making of experimental model for determining efficiency of acupuncture and analogous treatment application in allogenic rhinoplasty.

Materials and methods

Experiment was performed on 36 rabbits "Chinchilla". Line slit of nasal bone, moving under periosteum of first sterilized ear allogenic transplant taken from an other animal were performed under intravenous anesthesia of thiopentali-natrium (0,2 mg/kg) and local infiltration anesthesia (Novocaini 0,5% - 5 ml). Allogenic transplant was sterilized and conserved first with water Sol. Farmalini 0,5% within 3 days. The wound was closed in layers with atraumatic needle and materials (vicrilum). Animals were divided into 3 groups. Acupuncture stimulation of the acupoint GI4 was applied for the animals of the 1-st group. Local acupoints in the nose bone region were stimulated for the animals of the 2-nd group. Acupoint GI14 was irritated as well as local acupoints closed to the region of postoperative wound for the animals of the 3-rd group. Acupuncture treatment course have been lasted 10 days. Strong brake method has been applied for acupuncture irritation.

Results

Tissue infiltration in postoperative scar region of the 3-rd group animals was authentically less expressed five days postoperatively in respect of 1 and 2 groups ($p<0,01$ and $p<0,01$ correspondingly). That gives to take conclusion that to make experimental model of determining efficiency of acupuncture and analogous treatment application in allogenic rhinoplasty it is advisable to use acupuncture treatment model applied for the 3-rd group animals.

HIGH- STRENGTH BIORESORBABLE POLYLACTIDE FIBERS - PRODUCTION AND PROCESSING

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[Engineering of Biomaterials, 37, (2004), 9-10]

Introduction

Standard in surgical treatment of bone fractures is the use of metallic implants made out of bulk material with well defined mechanical characteristics, but without the ability to be resorbed nor to be radiolucent. A new generation of implants could, however, be seen as implants made of composite structures, either made of a single material or multiple materials, made of resorbable polymeric structures. This requires that these composite implants have mechanical properties close to, or equal to those of to date used metallic implants. Methods used in the production of metallic implants, as forging, casting or machining, however, are not well suited for the construction of bioresorbable composite implants. These problems could potentially be solved if highly oriented bioresorbable polymeric fibers are transformed into high-strength composite implants. The objective therefore is, to produce high - strength monofilaments made of resorbable polymers or co-polymers. Those monofilaments would then be used as basic building blocs for the construction of implant - structures.

Batch #	Polymer	Nozzle Ř [mm]	Nozzle temp [ºC]	Fiber Ř [mm]
1	poly(L/DL)	0.5	203	0.40 ± 0.05
2	poly(L/DL)	0.3	205	0.22 ± 0.02
3	poly(L/DL)	0.5	214	0.62 ± 0.03
4	terpolymer	0.5	227	0.55 ± 0.08
5	terpolymer	0.5	227	0.50 ± 0.05

TABLE 1. Extruder parameters.

Materials and methods

Polymers

Polymers used in the study were poly(L/DL-lactide) 80/20% with a viscosity-average molecular weight of 160.000 dalton (PURAC CCA, Holland) and a terpolymer with a viscosity-average molecular weight of 370.000 dalton developed in our institution, based on L, DL-lactides-glycolide (PURAC CCA, Holland). Both polymers were dried to a constant weight under vacuum before melt extrusion into monofilaments with diameters between 0.2 and 0.65 mm (Brabender, Plasticorder PL 2100) (TABLE 1). The monofilaments were then subjected to hot-drawing.

Hot-drawing device

Hot drawing of the monofilaments was done with a custom made tool, equipped with feed roller, heater and drawing roller (FIG.1). The feed roller had a range of 0.25 m/s-1.0 m/s and the drawing roller a range of 1.85 m/s-8.95 m/s. The resultant draw ratio therefore was between 1.85 and 35.8 respectively. The temperature range of the heater was between 50ºC and 175ºC. The different rollers and the heater were microprocessor- controlled with an accuracy of ± 0.025 m/s of the rollers and ± 0.2 ºC of the heater. The drawing zone had a total length of 210 cm with a heater zone of 6 cm in the middle. The relaxing zone was 13 cm and the takeup speed of the bobbin was equal to the speed of the drawing roller. The fibers were drawn with different draw-ratios and heater- temperatures (see TABLE 2).

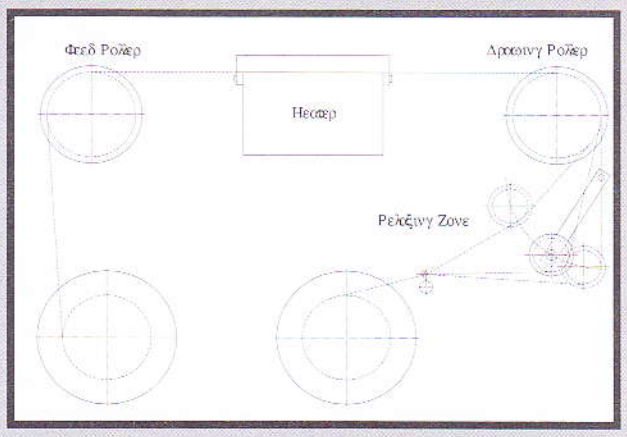

FIG. 1. Hot drawing device.

Testing

After treatment, the fibers were tested for their mechanical properties as tensile strength and Young's Modulus. The tests were done with a commercial test- rig (Instron model 4302 tester, High Wycombe, Bucks, England). The sam-

Batch #	Polymer	Draw ratio	Initial fiber Ř [mm]	Drawn fiber Ř [mm]	Heater temp. [oC]	Initial strength [MPa]	Strength drawn [MPa]	Modulus [MPa]
1	(L/DL)	8	0.4±0.05	0.18±0.02	135	62±2	549±25	6151±466
2	(L/DL)	8	0.22±0.02	0.1±0.02	125	61±4	692±65	7040±650
3	(L/DL)	9	0.62±0.03	0.26±0.04	155	50±18	595±60	6080±471
4	terpolymer	10	0.55±0.08	0.15±0.02	135	67±17	591±56	6630±1170
5	terpolymer	10	0.50±0.05	0.19±0.05	135	57±2	553±52	6560±560

TABLE 2. Hot-drawing conditions and mechanical parameters

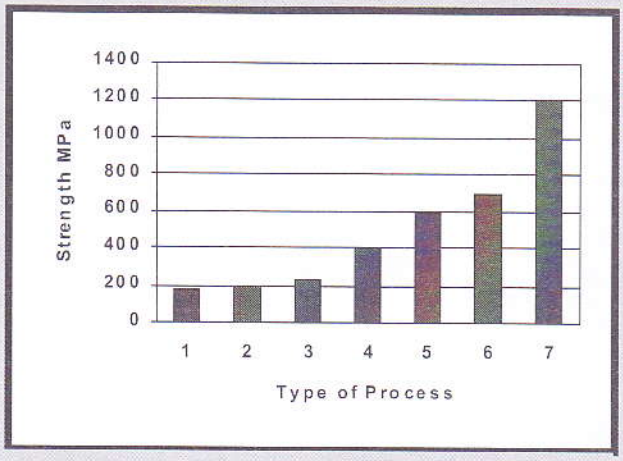

FIG. 2. Strength and elastic modulus of different monofilaments

Process	oung's Modulus [GPa]
1: Injection molding	4
2: Solid state extrusion KIST	8.4
3: Solid state extrusion SF & SG	14
4: Maxon monofilament	0.9
5: Author monofilament batch 3 & 4	6
6: Author monofilament batch 2	7

ples were clamped with fiber clamps with a grip distance of 100 mm. The load cell used had a range of 0.1 kN and an accuracy of 0.001 kN. The crosshead speed was 20 mm/min. The tests were carried out using three samples of each material and the average of the three values was used for further data- processing.

Results

Monofilaments with a tensile strength of >500 MPa and a Young's Modulus of >5500 MPa could be produced by hot- drawing of the melt- extruded material when drawn with a draw ratio of 8...10 and temperatures between 125 and 155°C. These conditions guarantee a continuous monofilament production. The strength of the monofilaments produced by the authors is more than twice of that reported in the literature (FIGURE 2).

Discussion

By hot-drawing, the fiber diameter was reduced by a factor of 2 to 2.5. To manufacture thin fibers, the initial fiber diameter needs therefore to be kept low. The limiting factors however are the processing parameters of the extruder, like nozzle diameter, pressure and temperatures. The strength of the monofilaments can further be increased if the thermo- oxidative degradation upon melt extrusion is diminished. The high- strength, high- modulus polylactide monofilaments can be transformed into high- strength internal fixation devices by solvent welding [8].

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EXPERIMENTAL MODEL FOR STUDYING EFFECTIVENESS OF TREATMENT AND PROPHYLAXIS PROCEDURESS ON THE BEGINNING AND DEVELOPPING OF PURULENT-INFLAMMATORY PROCESSES

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[Engineering of Biomaterials, 37, (2004), 10-11]

For many years the problem of purulent-septic *complications* stays one of actual as for clinics of cranio-maxillofacial surgery as well for other stomatological and surgical clinics. Nature of purulent complications evaluation has been changed within last years. Very often we meet hard forms of purulent infections when purulent process is located in some anatomic regions and have severe complications which cause danger for human life. That situation makes to elaborate new preventive, treatment and prophylaxis procedures, so there is necessity to do medico-biological examinations to appreciate effectiveness of some treatment procedures and to compare them.

Aim

of this research is to elaborate experimental models for studying effectiveness of treatment and prophylaxis procedures on the beginning and development of purulent-inflammatory process.

Materials and methods

Guinea pigs have been subjected into the experiment which has been performed on 21 animals of the same weight and age. Animals have been operated according to the same

schema: under the local anaesthesia Novocaine 0,25% - 5-7 ml, we have cut animals back hair on the area of 5,0 x 5,0 cm. The operation field was treated with an antiseptic (solution spirituous tincture of iodine). We have done two incisions of 3,5 cm. length of the parallel of vertebral spinal skin and subcutaneous fibrous tissue. The subcutaneous fibrous tissue was separated from the muscular fascia. The culture of variety field of St. aureus (in concentration 1:500 and 1:1000) was leaded into the wound. On the wound it was put postpone knot stitches with an atraumatic suture material (vicrilum - 5 - 0). The animals were assessed at 24, 48 hours, 3, 7, an 14 day, and followed up 3 and 6 months postoperatively.

Results

of experimental studies had demonstrated development of purulent-inflammatory process in 100% of cases.

Conclusions

All mentioned above gives to make conclusion that described method of making experimental model for studying effectiveness of treatment and prophylaxis procedures on the beginning and development of purulent-inflammatory process is simple to be reproduced, it does not demand a lot money. That is why it is to be recommended for large use in medico-biological investigations.

METHODS OF EXPERIMENTAL MODELS MAKING TO STUDY STIMULATION OF REGENERATION PROCESS OF MANDIBLE BONE TISSUE

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[Engineering of Biomaterials, 37, (2004), 11-12]

Traumatic fractures of mandible is one of actual problem in cranio-maxillofacial surgery. Increasing number of maxillofacial injures is involving rise of traumatic fractures of mandible last years, which is varying from 67,4% to 85% [...]. It is well known frequency of adult people dental anomaly makes from 33,6% to 63%. That fact bring grate interest of specialists to look for new and more effective methods of treatment and surgical procedures of mentioned above diseases. It requires making of new experimental models to study stimulation of regeneration presses of mandible bone tissue.

Aim of research

is to elaborate new experimental models for studying stimulation of regeneration processes of mandible bone tissue which meet the following requirements: 1) operation procedures are to be done painless; 2) experimental animal is to be kept alive postoperatively with good function of dental activity.

Materials and methods

Experiment was performed on 24 dogs of the same weight and age. Operation has been done under intravenous anesthesia with Sol. Thiopentali-Natrium 10%, 40 - 45 mg per 1 kg of animal weight. Using of that anesthesia treatment has permitted to make operations on mandible within 1,5 - 2 hours without additional anesthesia. They have used approximately 15 ml of Sol. Thiopentali-Natrium 10% while one operation procedure. That method gives to avoid complications as during operation procedures as well after it.

Operations have been performed in aseptic conditions.

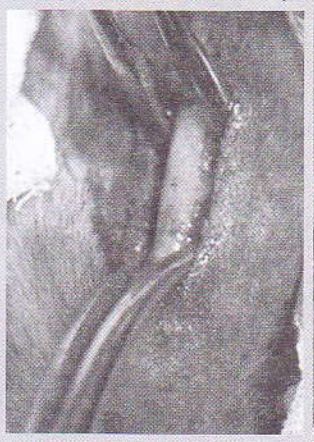

FIG. 1a) skeletized part of the horizontal section of animal mandible.

FIG. 1b) osteotomic cut of the horizontal section of animal mandible.

Episodes of operation procedures are shown on the FIG.1. Incision has been made parallel in 1 sm to the edge of mandible. Skeen was cut till the bone. After the periosteotomy and skeletization of horizontal part of mandible made by SIEMENS stomatologic equipment, osteotomy has been performed under the angle of 80 - 90in the region of 5-6th tooths. Nerves and capillary have stayed undamaged as it is shown in the FIG.2. Tooths of osteotomical region have been extracted. After the operation 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%. Than the same operation has been made on the opposite site of animal mandible. Postoperatively, all animals had antibacterial and anti-inflammatory treatment course of 7 days. Animals were treated by Sol. Lincomicini 30% - 1 ml, Sol. Analgini 50% - 2 ml. once per day intramusculary.

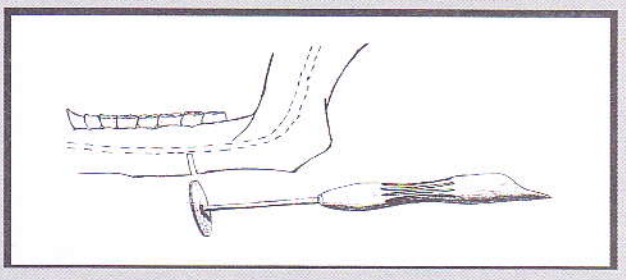

FIG. 2. Schema of osteotomical cut for experimental model of mandible trauma.

In order to take samples for histological examination, animals were subjected into intravenous anesthesia. Samples have been taken 7, 14, 21 days and 1,2 months later. Earlier damaged bone of mandible was sawn for samples. It was a piece of bone with 4 sm of width, 2 sm lengh from line of cut to each side. This hollowness has been filled with active materials of "biossetal" [O.P.Chudakov, A.M.Grehukha, A.Z.Barmutskaya et all, 2002]. They have put stiches in a wound slit by slit. After the experiment has been finished, samples have been put into the Sol. Farmalini 10%. Decalcinated by HNO_3, they have been placed into alcohol and put into paraffin finaly. Misroscopic sections have been painted according to the methods of Van-Guison and put into balm. Histological preparations have been made for latest examination in details and studying with light microscope.

Conclusion

Described above methods of making of experimental models to study processes of bone tissue regeneration of mandible are different from old ones. It allows to keep animals alive, there maxillofacial system stays in normal conditions. In some times these animals could be used for new experiments.

APPLICATION OF THE NEW BONE-REPLACING MATERIAL "KAFAM" IN STOMATOLOGY

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[Engineering of Biomaterials, 37, (2004), 12-13]

Treatment of the patients suffering from chronic periapical destructive processes, chronic complex periodontitis, benigh formations of the maxillae and other diseases of facial bones is the important and urgent problem of maxillofacial surgery since the above-mentioned diseases are very often encountered in the practice of physician-dentists. According to the WHO data obtained from examining the population of 53 countries the prevalence of periodontitis diseases attains 98% [1]. Among the grown-up population of the Republic of Belarus is practically the

100% morbidity of gingivity, simple and complex periodontitises [2, 3]. The results of the last two decades are evident of the fact that chronic apical periodontises are 30% and benigh tumors and cysts are no less than 45% of the total number of surgical maxilla diseases. In the majority of cases the immediate and distant results on the treatment of patients with such affections remain unsatisfactory [4]. At the same time in the USA, Germany, Great Britain, Switzerland, Japan and in other countries new osteoplastic materials are actively used for treating the above diseases. These materials promote recovering bone tissue, enable one to stop destructing the tooth root and to stimulate the processes of purposeful regeneration and reconstruction of the tissues of living organism. Calcium phosphate materials [5] are used in modern maxillofacial surgery. In Russia bone defects are filled with home-produced synthetic hydroxyapatite-based materials: "Ostim-100", "Gapkol", "Kolapol", "Kollapan", etc. [6].

In the Republic of Belarus the co-workers at the Institute of General and Inorganic Chemistry of the NAS of Belarus and at the Maxillofacial Surgery Chair of the Belarusian State Medical University are also carrying out investigations on creating osteoplastic materials. Unlike the Russian identical materials, Belarusian porous ceramics "Kafam" is stable in shape. When mixed with the blood in the operative wound, it makes a porous structure needed for a further purposeful growth of the cells of the newly formed bone tissue.

The chemical composition and structure of the developed material are adequate to those of the mineral part of the human bone. The calcium-to- phosphorus ratio is within 1.67-1.70 and corresponds to the one in the human native bone. This material can be sterilized many times, not losing its properties and can be used in combination with different-type antibiotics and antiseptics. The application of "Kafam" in medical practice does not require special instrumentation and equipment. It is produced in different shapes (blocks, plates, granules from 0.1 to 1.2 mm) in four types A, B, C and D that differ by heat treatment temperature and strength. All-type materials are used for surgical treatment of different stomatological diseases [7]. This material underwent technical, sanitary-hygienic, biomedical and clinical tests. Its use in stomatological practice was supported by the permission of the Ministry of Health of Belarus IM. 7. 3743 of March 20, 2003.

In planning the surgical intervention it is necessary to choose a required shape of "Kafam" - blocks, plates or granules. The size and shape of the implant material are chosen individually for every patient depending on the size and the shape of the bone defect. To illustrate the application of the material "Kafam" in stomatological surgery, two methods are presented below.

Procedures of filling the operative bone defects after radicular cysts are removed (Fig. 1). Prior to the operation the calcium phosphate ceramics "Kafam" undergoes sterilization together with operative instruments. A patient is tested on the sensitivity to a used antibiotic. The radicular cyst is removed using the traditional surgical methods. The formed bone defect is instilled with an aqueous 0.05% chlorhexidine solution and then with a 30% dichloride lyncomicine solution (FIG. 1a). The defect cavity is loosely filled with "Kafam" granules (type A, 0.5-0.6 mm in size) with a surgical spoon (FIG.1b). Having been instilled with the blood the granules form a blood clot with the material introduced (FIG. 1c). A mucoperiosteal graft is returned to its place and is fixed with separate interrupted sutures (FIG. 1d). Sutures are removed after 6-7 days. A patient is then under dynamic observation.

Procedures of treating periodontitis (FIG. 2). After the granulation tissue and tooth deposits are removed, the tooth roots are polished and also the alveolar bone border and the inner surface of the mucoperiosteal graft are treated. The wound is treated with an aqueous 0.05% chlorhexidine solution. If a patient is very sensitive to antibiotics, then the instillation with a 30% dichloride lyncomicine solution is not made. After the operative wound has been visually examined and the sizes of the marginal periodontium defect have been determined, the "Kafam" plate (type C) of the corre-

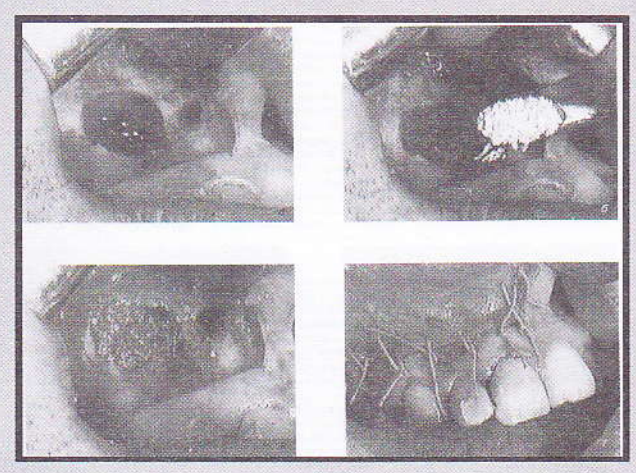

FIG. 1. The filling of bone defects after the radicular cyst removal.

sponding shape and size and "Kafam" granules (type B, 0.2-0.3 or 0.3-0.5 mm in size) are chosen. The plate is introduced into the periodontal pocket. Slightly pressing it the defect is filled. As the plate can be loosely adjacent to the walls of the bone defects, granules are additionally introduced into the pocket. The mucoperiosteal graft is returned to its place and is sutured. Then the protective gum dressing is placed.

Sutures are removed after 7-8 days.

During the postoperative period the patients with the

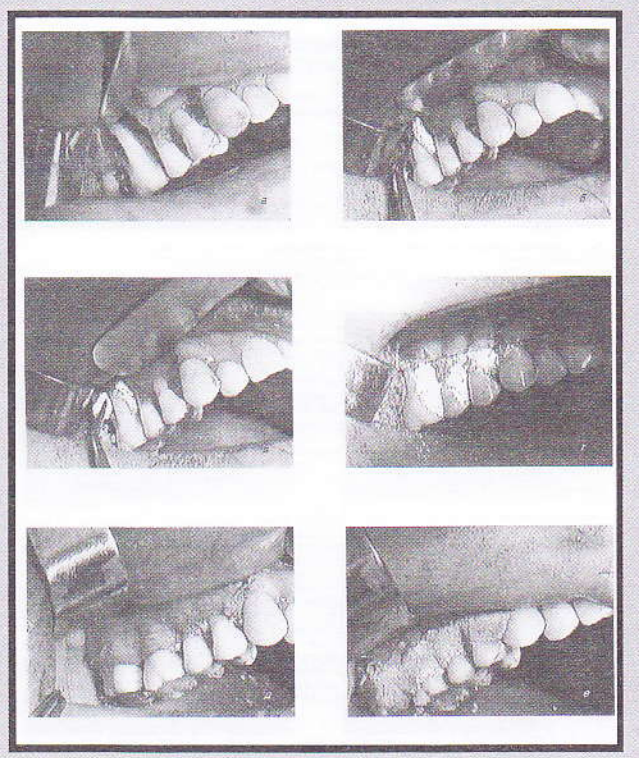

FIG. 2. Gingivioplastics using the material "Kafam".

bone defects filled with "Kafam" were subjected to X-ray examinations. Small operative defects that are formed after the cyst removal were filled with a young bone regenerate irrespective of the used material type and the granule size in 2-3 months. As for the patients with medium and large postoperative bone defects, the decrease in bone defect sizes was seen in 3 months. In this case, the regeneration process was more clearly observed at its periphery. After 6 month the mature bone tissue was found using X-rays at the places of small and medium defects. As for the patients with large postoperative bone defects the complete regeneration of the mature bone tissue was observed after 1 year.

As for the patients operated upon chronic complex periodontitis, in 3 months after treatment the stable remission was seen, the haemophilia stopped, the tooth mobility diminished, and the depth of periodontal pockets decreased to 3 mm. The X-ray examination revealed the formation of new bone structures, the osteoporosis reduction and the improvement of the sharpness of bone contours. In total, more than 200 operations were made using the material "Kafam".

The positive immediate and distant results on the surgical treatment of benigh formations of maxillae and also of chronic complex periodontitis, when posoperative bone defects are filled with calcium phosphate ceramics "Kafam", permit one to recommend this material for use in everyday stomatological practice.

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THE INFLUENCE OF CHEMICAL STRUCTURE OF ALIPHATIC POLYESTERS ON ADHESION AND GROWTH OF OSTEOBLAST-LIKE MG63 CELLS

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Abstract

Degradable copolymers of glycolide and L-lactide (PGLA), glycolide and ε–caprolactone (PGCap) and terpolymer of glycolide, L-lactide and ε–caprolactone (PGLCap) were synthesized by ring opening polymerization using zirconium acetylacetonate ($Zr(acac)_4$) as a biocompatible initiator. The structure and physicochemical surface properties of the materials were studied by NMR spectroscopy, gel permeation chromatography, X-ray photoelectron spectroscopy and sessile drop. The interaction of polymeric films produced by slip-casting with osteoblast-like MG63 cells was tested in vitro. The number of adhering cells, their shape and the size of cell-material contact area were evaluated from day 1 to 7 after seeding. It was found that the cell behaviour on PGLA and PGLCap was very similar as on control tissue culture polystyrene (TCPS). On PGCap, however, the number of initially adhering cells was significantly lower (by 84% than on TCPS) and cell spreading area smaller (by 50% than on TCPS). On day 7 after seeding, these cells reached the lowest population density (by 30% smaller than on TCPS). We hypothesize that the lower cell adhesion and growth of MG63 cells on PGCap was caused by the highest hydrophobicity of this material among all studied samples.

[Engineering of Biomaterials, 37, (2004), 14-17]

Introduction

Aliphatic polyesters such as polylactides, polyglycolide, poly-ε-caprolactone and their copolymers have been widely used in medicine as materials for sutures, prostheses or implants for internal fixation of bone fractures [1]. More recently these polymers have received considerable attention as carriers for controlled release of drugs and biodegradable scaffolds for tissue engineering [2].
It was recently shown that such materials can be synthesized by the use of initiators of lower toxicity, instead of commercially used, but highly toxic tin compounds. These initiators are represented by compounds of zinc, calcium, iron and zirconium [3-6].

Extensive studies of aliphatic polyesters over past two decades have shown that surface chemistry, wettability, topography and roughness markedly influence biological properties such as protein adsorption, cell attachment, spreading and proliferation, ultimately affecting new tissue formation [1, 2, 7, 8].
In the present study we characterize chemical structure and surface properties of three aliphatic polyesters synthesized with the use of zirconium acetylacetonate, and investigate the behaviour of osteoblast-like MG 63 cells contacting these materials in vitro.

Materials and methods

Synthesis

Preparation of substrata was described in detail earlier [6,9]. Briefly, copolymerization of glycolide and L-lactide (Purac, Netherlands) (PGLA), glycolide and ε- caprolactone (Fluka, Germany) (PGCap) and terpolymerization of glycolide, L-lactide and ε-caprolactone (PGLCap) were performed in bulk with a zirconium (IV) acetylacetonate $Zr(acac)_4$ (Aldrich Corp., Germany) molar ratio of 1.25×10^{-3} at 100°C by a conventional method using a vacuum line for degassing and sealing of the ampoules. The resulting materials were ground and shaken with methyl alcohol in order to remove non-reacted monomers and then dried in vacuum at 50°C.

The films were cast from 10% (w/v) polymer solution in methylene chloride on glass Petri dishes, followed by vacuum drying for 72h.

Measurements

The compositions of terpolymer and copolymers were determined by 1H NMR (Varian Unity Inowa spectrometer) at 300 MHz and a 5-mm sample tube. Dried dimethyl sulfoxide-d6 was used as a solvent. The molecular masses and polydispersity indices were determined by gel permeation chromatography (GPC) with the aid of Spectra Physics SP 8800 chromatograph (chloroform was used as the eluent, flow rate, 1 mL/min, Styragel colums 104, 103 and 500A and a Shoedex SE detector). Surface chemical composition was studied by X-ray photoelectron spectroscopy (XPS) (SSI X-Probe spectrometer, Surface Science Instruments, Mountain View, CA, USA) according to the method described previously [10]. The contact angle was measured by sessile drop method by an automatic drop shape analysis system DSA 10 Mk2 (Kruss, Germany). UHQ-water (produced by Purelab UHQ, Elga) of resistivity - 18 MΩ/cm, was used for experiments.

Cell culture conditions

The polymer samples were placed into Nunclon Multidishes (24 wells with diameter of 15 mm, Nunc, Denmark) and sterilised with ultraviolet irradiation for 1h from both sites. The MG63 osteoblast-like cell line (European Collection of Cell Cultures, Salisbury, UK; passage 156) were seeded on the foils at the initial density of 25,000 cells/cm^2 (i.e. 45,000 per well) in 1.5 mL of Dulbecco-modified Eagle Minimum Essential Medium supplemented with 10% foetal bovine serum and gentamicin (40μg/mL). Nunclon TCPS wells were used as control material. For each ex-

perimental group and time interval 2 samples were used. The cells were cultured for 1, 3 and 7 days at 37°C in humidified air atmosphere containing 5% of CO_2.

Cell adhesion and growth

The samples were rinsed with phosphate buffered saline (PBS), fixed in 4% formaldehyde in PBS for 5 min, stained with Gill's hematoxylin for 5 min, aqueous eosin Y for 2 min, and mounted in Glycerol Gelatin (all chemicals provided by Sigma Diagnostics, U.S.A.). The number of adhering cells on day 1 was counted under phase-contrast microscope (Opton, Germany) in 10 randomly selected microscopic fields of 1 mm^2 homogenously distributed on each sample. On day 7, because of high cell population density, the cells were detached by trypsin-EDTA (Sigma) and counted in Coulter Particle Counter (Coulter Electronics LTd, Florida, U.S.A.; 3 measurements for each sample). For evaluation of cell shape and spreading area, images from 5 to 6 regions on samples 1 and 3 days after seeding were captured by Olympus IX 51 inverted microscope equipped with digital camera DP 70 and DP Control Software (objective x20, captured area of 1.376 mm^2). Atlas Tescan Digital Microscopy Imaging (Tescan Co., Brno, CR) was used for the analysis of cell area (13-50 cells per sample). Cells forming cell-cell contact were excluded from the evaluation.

Data were presented as averages ± SEM (Standard Error of Mean) from 6 to 50 measurements obtained from 2 independent experiments. The statistical significance of the differences was evaluated by the Student's t test for unpaired data and by one-way analysis of variance (ANOVA) using SigmaStat software (Jandel Corp., U.S.A.).

Results and discussion

Properties of substrata

The physical and chemical properties of the newly constructed biomaterials are provided in TABLE 1. The obtained data indicate that synthesised materials have different chemical structure, but quite similar molecular masses (Mn). Their surface composition, as determined by XPS, reveals that the highest amount of oxygenated functions (about 40 mole%) was detected for PGLA and PGLCap. On the other hand, only 27 mole% of oxygen was measured on the surface of PGCap. These results go along with the values of contact angles: the lowest contact angles, slightly above 70°, were measured on PGLA and PGLCap, contrary to PGCap which had contact angle of about 80°.

Cell culture

The morphology of MG63 cells adhering to examined substrata on 1, 3 and 7 days after seeding is shown on FIGURES 1, 2 and 3. On day 1, the cells on all samples, except PGCap, were mainly spindle-shaped and partially

Sample	N	Mn	d	S[d] mole%		θ
	mole %	kDa		C	O	deg
PGLA	18:82[a]	34	2.5	58	42	72.9±2.6
PGCap	9:91[b]	53	1.8	73	27	80.6±2.3
PGLCap	10:70:20[c]	48	1.3	62	38	71.7±3.4

N – molar ratio of [a] glycolide to L-lactide, [b] glycolide to ε-caprolactone and [c] glycolide to L-lactide and ε-caprolactone; Mn – number average molecular mass; d – polydispersity coefficient (Mw/Mn); [d] surface composition excluding hydrogen - studied by XPS; θ – contact angle

TABLE 1. Properties of substrata

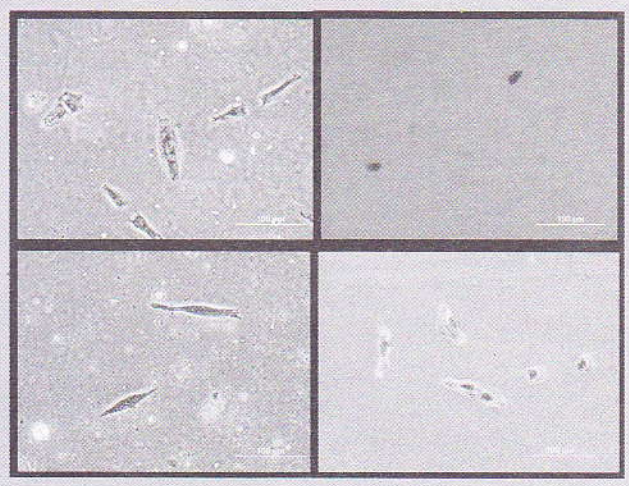

FIG. 1. Morphology of MG63 cells adhering to PGLA (A), PGCap (B), PGLCap (C), and Nunclon TCPS (D) on day 1 after seeding. Hematoxylin - eosin staining. Olympus IX51 inverted microscope with DP Controller software, objective 20x, bar 100 μm.

spread, while on PGCap, the cells were round and non-spread [FIG. 1]. The spreading area of cells on day 1 after seeding was also significantly smaller on PGCap in comparison to TCPS, PGLA and PGLCap [FIG. 4].

Similarly, in 3-day-old cultures on PGLA, PGLCap and TCPS, the cells were more flattened, mainly polygonal in shape. On the other hand, on the PGCap the cells were less spread [FIG. 2]. On day 7, the cells on all substrata formed monolayers [FIG. 3].

The number of cells adhered on PGLA and PGLCap on day 1 and 7 after seeding [FIG. 5 and 6 respectively], was comparable to that on TCPS and significantly higher than that on PGCap.

The lower cell adhesion and subsequent growth of MG63 cells on PGCap could be explained by a relatively high water contact angle found on this substrate, which is a sign of its hydrophobicity. Similar results were obtained on human dermal fibroblasts and myoblasts, Baby Hamster Kidney (BHK) cells or vascular endothelial cells cultured

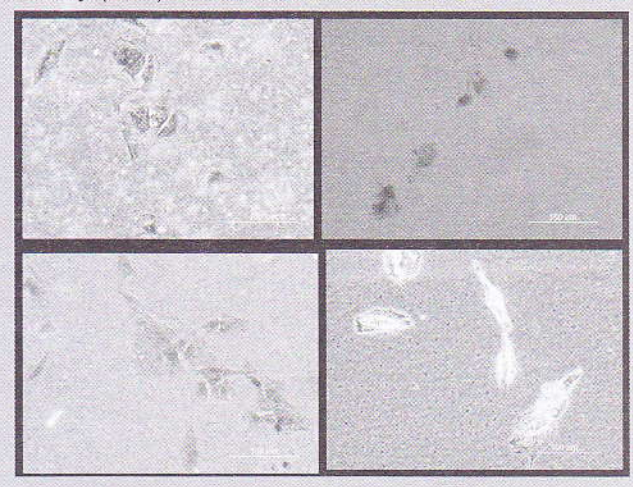

FIG. 2. Morphology of MG63 cells adhering to PGLA (A), PGCap (B), PGLCap (C), and Nunclon TCPS (D) on day 3 after seeding. Hematoxylin - eosin staining. Olympus IX51 inverted microscope with DP Controller software, objective 20x, bar 100 μm.

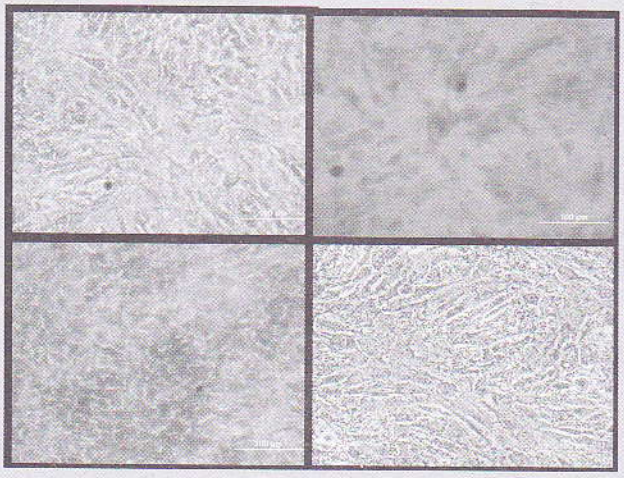

FIG. 3. Morphology of MG63 cells adhering to PGLA (A), PGCap (B), PGLCap (C), and Nunclon TCPS (D) on day 7 after seeding. Hematoxylin - eosin staining. Olympus IX51 inverted microscope with DP Controller software, objective 20x, bar 100 μm.

on pure poly-ε-caprolactone or its copolymers with poly(L-lactide) or poly(ethylene glycol) (PEG). When the hydrophilicity of these materials was enhanced by physical and chemical surface modification, such as treatment by plasma discharge, polymerization with acrylic acid, hydrolytic etching or increased content of PEG, the colonization of these substrates with cells markedly improved [11-13].

The surface wettability of polymer samples in this study was positively correlated with the concentration of oxygen-containing chemical functional groups on the material surface, which are well known to support adhesion, growth and differentiation of various cell types [14]. The increase of the material surface wettability is probably not the only mechanism by which these groups improve the cell adhesion on biomaterials. In our earlier study [14] the number of vascular smooth muscle cells initially adhered to polyethylene implanted with O+ or C+ ions cells appeared to be positively correlated with the amount of the oxygen group present at the polymer surface rather than with the surface hydrophilia arising from other reasons. Oxygen groups may have a direct influence on adsorption of cell adhesion-mediating extracellular matrix molecules (e.g., fibronectin, vitronectin, collagen provided by the serum in the culture media) to the materials [14].

Other important surface features, which might influence the cell adhesion on our polymers, were the surface roughness and topography. It has been reported that nanostructured surfaces, i.e. surfaces with nanometer features, e.g. grains, markedly promoted cell adhesion, which was explained by adsorption of cell adhesion-mediating extracellular matrix proteins in appropriate conformation allowing their good accessibility by adhesion receptors on cells [15].

Conclusions

The number and spreading of MG63 cells, comparable to that on control TCPS, was found on foils made of PGLA and PGLCap. On the contrary, significantly lower

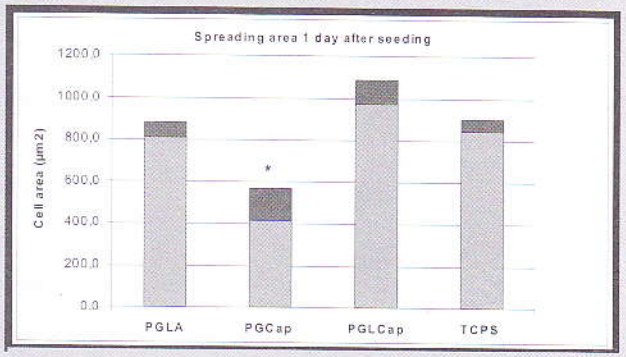

FIG. 4. Spreading area of MG63 cells adhering to PGLA, PGCap, PGLCap, and Nunclon TCPS on day 1 after seeding. Averages +/- SEM from 13 to 50 measurements on two independent samples; Student's t-test for unpaired data, *P<0.01 compared to TCPS.

number of cells, different cell morphology and smaller spreading area were observed for PGCap.

Such differences are likely due to much higher hydrophobicity of PGCap surface, resulting from lower content of surface oxygen. The correlation between physico-

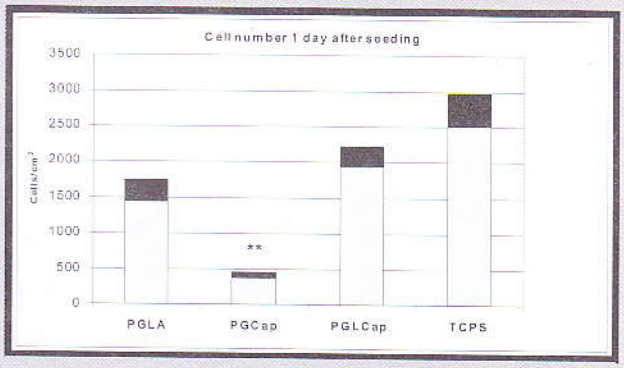

FIG. 5. Number of MG63 cells adhering to PGLA, PGCap, PGLCap, and Nunclon TCPS on day 1 after seeding. Averages +/- SEM from 20 measurements on two independent samples; Student's t-test for unpaired data, **P<0.001 compared to TCPS.

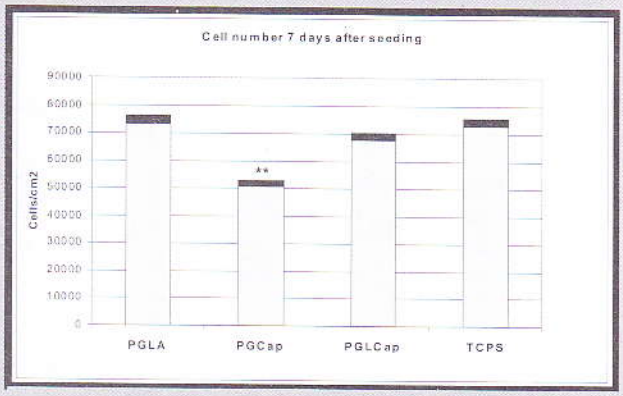

FIG. 6. Number of MG63 cells adhering to PGLA, PGCap, PGLCap and Nunclon TCPS on day 7 after seeding. Averages +/- SEM from 6 measurements on two independent samples; Student's t-test for unpaired data, **P<0.001 compared to TCPS.

chemical surface properties and cell behaviour, provided by the present study, may help in better understanding of the phenomena at the interface of a biomaterial and its biological environment. Moreover, it may provide some idea how to modify cell behaviour by simple changing the material chemical composition.

Acknowledgements

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INFLUENCE OF SURFACE PROPERTIES OF CARBON BASED MATERIALS ON CELL SPREADING

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Abstract

Carbon materials are generally well tolerated by animal cells. The possibility of applying carbon fiber reinforced carbon (CFRC) composite materials is given by their excellent biocompatibility and porosity, coupled with a modulus which can be tailored to be similar to that of bone. This makes them an attractive material for bone plates and implants in orthopaedic and dental surgery. It is known that the volume properties of a material usually have little or no influence on the surrounding living tissue cells. In general, biocompatibility is controlled mainly by the interface between biomaterial and living tissue cells.

The literature and our study indicate that the interaction at the interface is specifically controlled by the surface morphology, (i.e., especially by surface roughness), and by the chemical state of the surface - by hydrophobia (wettability), free chemical bonds and present chemical groups, etc. Never-theless, biocompatibility can be improved by a suitable change of these parameters. There are several possible methods for influencing the roughness and chemical state of the surface. One way to change the surface properties is by preparing a suitable coating. The properties of the surface are controlled by process technology, and the grinding and polishing of the substrate can be used for roughness control.

Till now we studied the influence of the surface on the cell adhesion and on the rate of the cell growth. There, we have studied the influence of a surface coating of CFRC using a several types of layers on the base of carbon. In our present contribution we continue in this work using the surfaces of CFRC in native and polished states, both covered by layers of amorphous carbon, or titanium with carbon or pyrolitic graphite. The vascular smooth muscle cells were grown on these surfaces. The purpose of this paper is to find the influence of the surface on the important parameter of tissue cell growth - the spreading of cells.

The main topic of this work is therefore the measurement and statistical evaluation of the cell area on the various types of surfaces. It will be shown, that the cell spreading is strongly influenced by various surface roughness and also its chemical state.

[Engineering of Biomaterials, 37, (2004), 17]

VASCULAR ENDOTHELIAL CELLS IN CULTURES ON METAL/ C:H COMPOSITE FILMS

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Abstract

Adhesion, proliferation and maturation of vascular endothelial cells in cultures derived from the bovine pulmonary artery (line CPAE) were studied on Ti/C:H or Ag/C:H films prepared on glass using dc magnetron plasma deposition. The concentration of Ti or Ag in the films ranged from 0% to 20%. On Ag/C:H layers, increasing concentration of silver markedly decreased cell viability. Only samples with very low silver content allowed cell spreading and formation of continuous endothelial layer. Although long-term presence of this material in patient's organism would not be advantageous, antimicrobial effect of silver may be favorable for short term applications, e.g. coating of catheters. On Ti/C:H layers, the cells were similarly or more active in adhesion, proliferation and maturation than those on glass or pure amorphous carbon, respectively. This material could be suitable for long-term use in both soft and hard tissue surgery, e.g. for inner coating of vascular prostheses or surface modification of artificial bone implants.

[Engineering of Biomaterials, 37, (2004), 18-20]

Introduction

Amorphous hydrogenated carbon (a-C:H, also called diamond-like carbon) has been used for surface modifications of various polymer- or metal-based biomaterials in order to increase their wear resistance, smoothness, hydrophobia and blood compatibility [1-5]. For example, it has been employed for coating of femoral heads of metallic hip prostheses in order to prevent the release of metal ions and wear particles [1] or deposited on various blood-contacting devices, e.g., blood pumps, in order to enhance their resistance to protein adsorption and thrombus formation [2-5].

The physicochemical properties of a-C:H films and their attractiveness for colonization with cells can be further modified by incorporation of metal ions into these layers [6, 7]. Thus, the aim of this study is to evaluate the adhesion, growth and maturation of vascular endothelial cells in cultures on Ti/C:H or Ag/C:H films deposited on glass. Titanium is widely used material for construction of bone, joint or dental implants, and it has been reported to support adhesion and growth of osteogenic and vascular smooth muscle cells [6, 7]. Silver is known by its strong antimicrobial activity [8, 9].

Material and methods

Ti/C:H or Ag/C:H films were deposited on glass using unbalanced magnetron with Ti or Ag target operated in the dc mode in a working gas mixture of n-hexane/Ar [10]. As estimated using transmission electron microscopy (TEM), atomic force microscopy (AFM), Rutherford backscattering technique (RBS) and elastic recoil detection analysis (ERDA), the concentration of Ti or Ag in the films increased proportionally to the amount of Ar in the working gas mixture, ranging from 0% to 20% [10].

The samples (size 8x8 mm) were sterilized by UV irradiation, inserted into Nunclon Multidishes (NUNC, Denmark; 24 wells, diameter 15 mm) and seeded with bovine pulmonary artery endothelial cells (line CPAE, ATCC CCL 209, 17 000 cells per cm^2). The cells were incubated in 1.5 ml of Dulbecco's Modified Eagle Minimum Essential Medium supplemented with 20% of fetal bovine serum for 1, 3 or 7 days at 37°C in air atmosphere with 5% CO_2.

Adhesion of endothelial cells on the metal/C:H films were evaluated by the number of initially adhering cells on day 1 after seeding, cell-material contact area (i.e., cell spreading area) and formation of focal adhesion plaques, detected by immunofluorescence staining of vinculin [11]. Cell proliferation was estimated by the percentage of cells newly synthesizing DNA. The nuclei of these cells were visualized by 30-min-incubation of cells with bromodeoxyuridine (BrdU), followed by anti-BrdU immunoperoxidase staining [11]. Markers of endothelial cell maturation, used in this study, were represented by formation of confluent cobblestone-like cell layer, content of von Willebrand factor and formation of distinct beta-actin cables. The latter two parameters were evaluated by immunofluorescence staining [11].

Results and discussion

On Ti/C:H layers, the endothelial cells adhered in higher initial numbers and by a large cell-material contact area than on pure amorphous carbon (FIGS. 1A, B). Similar results were obtained earlier on vascular smooth muscle cells, osteoblast-like MG-63 cells or bone marrow cells cultured on carbon-fibre reinforced carbon composites (CFRC) or titanium discs coated with Ti-containing a-C:H [6, 7]. The improved cell adhesion could be explained by formation of oxygen-containing groups in the Ti/C:H layers and their increased wettability [6, 7, 10]. The synthesis of DNA in cells on Ti/C:H samples, measured by BrdU incorporation, tended to be lower than on a-C:H, although these differences were not significant (FIG. 1C). The latter result is consistent with the findings that the proliferation activity is the highest at the intermediate strength of cell adhesion. When the adhesion is very high, the cells skip the proliferation phase and enter sooner the differentiation program [11]. Both a-C:H and Ti/C:H films allowed formation of continuous layers of cobblestone-like endothelial cells (FIG. 2A), although this formation was sooner on Ti/C:H. Thus, the Ti/C:H films showed a good compatibility with endothelial cells, so that these materials could be used for cardiovascular applications, such as coating of vascular prostheses or heart valves in order to improve their lining with endothelial cells. Another possible application is surface coating of bone implants.

On Ag/C:H layers, increasing concentration of silver

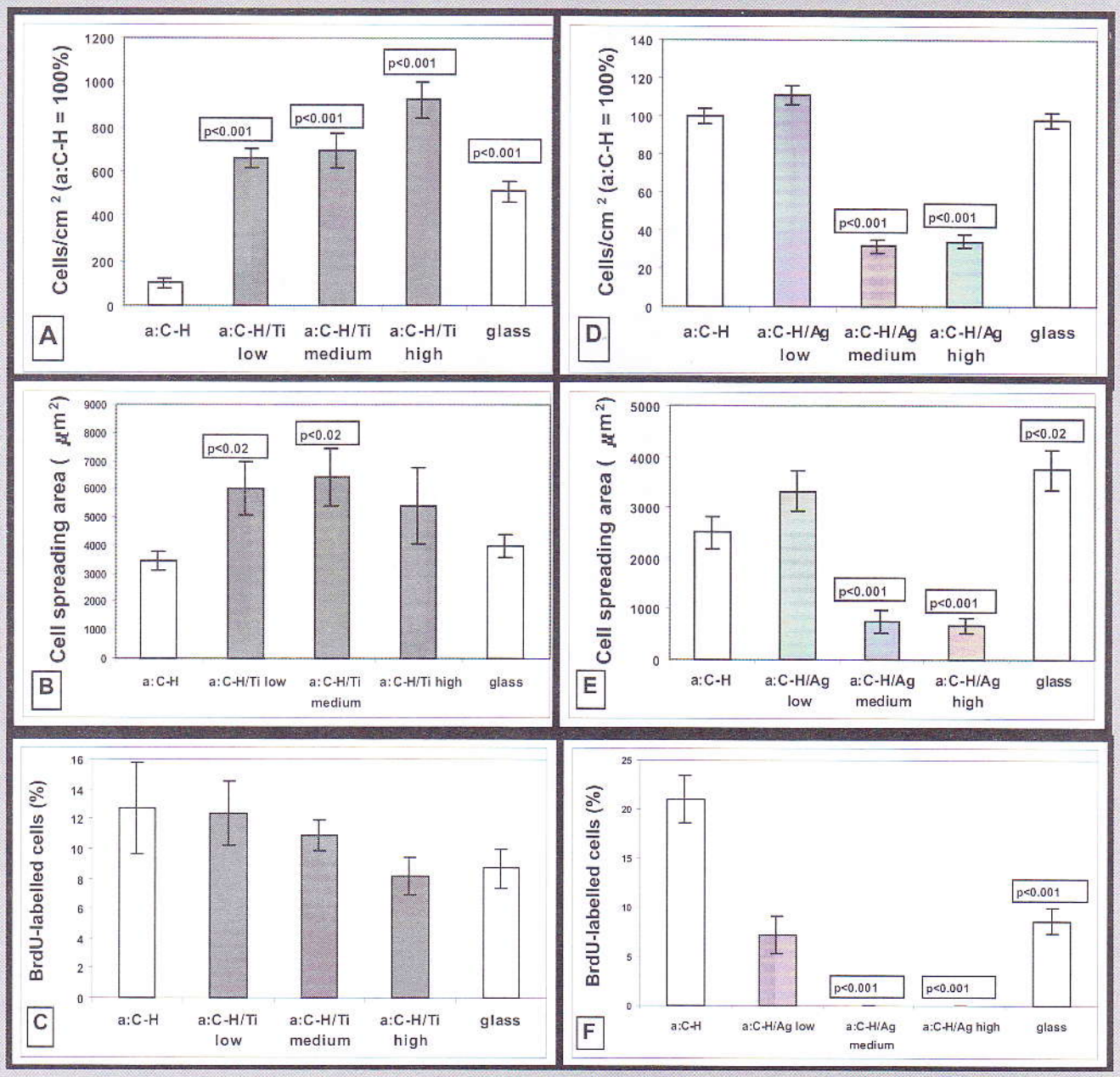

FIG. 1. Number (in % of values obtained on pure a:C-H; A, D), cell-material contact area (B, E) and percentage of DNA synthesizing endothelial cells (C, F) on a:C-H layers with low, medium and high concentrations (within the range approx. from 1% to 20%) of titanium (A-C) or silver (D-F) on day 1 (A, B, D, E) or day 3 (C, F) after seeding. Average ± SEM from 9-38 measurements, Student's t-test for unpaired data, statistical significance: p<0.02, p<0.001 in comparison with values obtained on pure a:C-H.

markedly decreased adhesion and proliferation of endothelial cells. Cells on the films with high and medium silver concentrations adhered at very low initial numbers and did not spread. They did not incorporate BrdU into DNA and usually died before day 7 of cultivation (FIGS. 1D-F, 2C). Only the layers with the lowest silver content allowed sufficient cell attachment and spreading, assembly of focal adhesion sites and actin cytoskeleton, synthesis of DNA and von Willebrand factor, and formation of continuous endothelial cell layer (FIG.1D-F, 2B, 2D-F). Similarly, polystyrene implanted with Ag- ions (energy from 5 to 30 keV, dose from 10^{14} to $6x10^{16}$ ions/cm^2) also supported growth of vascular endothelial cells [12]. Therefore, if the Ag/C:H layers with relatively low silver content ensure significant antimicrobial effects, which remains to be investigated, the use of these coatings for construction of durable implants into human body could be advantageous. The Ag/C:H layers with the higher and cytotoxic silver concentrations could be suitable only for short-term contact with patient's organism, e.g. as antimicrobial coating of intravascular or urinary catheters [8, 9].

Conclusions

Ti/C-H and Ag/C-H films with low Ag concentration supported adhesion, growth and maturation of vascular endothelial cells in cell culture conditions. These films could be used for construction of long-term artificial implants into human body, e.g. for coating of the inner surface of vascular prostheses or surface modification of bone implants.

Ag/C-H films with higher silver concentrations were cytotoxic and could be suitable for antimicrobial coating of intravascular and urinary catheters for short-term insertion.

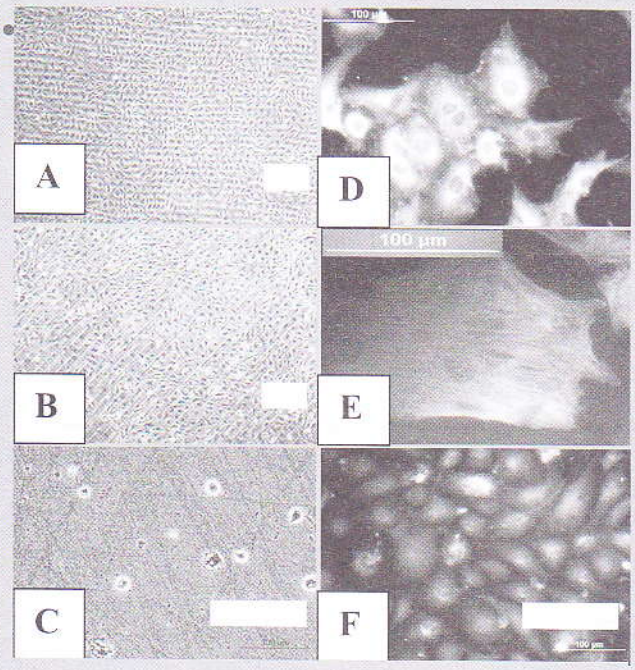

FIG.2. Morphology and molecular markers of adhesion and maturation of endothelial cells in cultures on Ti/C:H or Ag/C:H layers (concentration range of metals approx. from 1% to 20%). A, B: formation of a confluent cell layer on a-C:H with low concentration of Ti or Ag, respectively; C: dead cells on a-C:H with high concentration of Ag. D, E, F: immunofluorescence staining of vinculin-containing focal adhesion plaques, beta-actin

Acknowledgements

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We also thank Dr. Věra Lisá for maintaining the cell line CPAE in culture and Mrs. Ivana Zajanová for immunofluorescence staining.

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EVALUATION OF THE EFFECT OF TRANSPHYSEAL BIOABSORBABLE SCREWS ON GROWTH OF RABBIT FEMUR

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Abstract

A self-reinforced bioabsorbable poly-L-lactide/polyglycolide (SR-PLGA) 80/20 screw 2.0 mm in diameter was implanted in a transphyseal location across the distal growth plate of the right femur in 24 immature rabbits. Radiological evaluation revealed a mean shortening of 3.1 mm at 3 weeks (p=0.050), 11.1 mm at 6 weeks (p=0.001), 9.3 mm at 24 weeks (p=0.011), 9.0 mm at 48 weeks (p=0.009) and 12.6 mm at 72 weeks (p=0.002) compared with the intact contralateral femur. Growth retardation continued for 6 weeks postoperatively (3 versus 6 weeks, p=0.003), after which the bones grew normally up to 72 weeks (p=0.6). The duration of temporary growth retardation correlated with that of strength retention of the SR-PLGA 80/20 copolymer. These findings suggest that SR-PLGA 80/20 screws can be applied in transphyseal bone fixation. The use of bioabsorbable screws for temporary epiphyseodesis seems attractive but requires further study.

Keywords: *Femur, growth, rabbit, SR-PLGA*

[Engineering of Biomaterials, 37, (2004), 20]

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WPŁYW DODATKU MODYFIKOWANEGO BENTONITU NA WŁAŚCIWOŚCI NANOKOMPOZYTÓW AKRYLOWYCH

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Streszczenie

Współczesna protetyka stomatologiczna dysponuje szeroką gamą materiałów, które mogą być wykorzystane w rehabilitacji układu stomatognatycznego. Częste uszkodzenia płyt protez mogą jednak świadczyć o zbyt małej wytrzymałości mechanicznej tworzywa akrylowego, z którego są wykonane. W pracy omówiono próbę modyfikacji tego tworzywa krzemianem warstwowym (bentonitem), w celu poprawy wytrzymałości protezy.

Słowa kluczowe: *nanokompozyty akrylowe - wytrzymałość, modyfikowane bentonity*

[Inżynieria Biomateriałów, 37, (2004), 21-27]

Wprowadzenie

Tworzywo akrylowe stosowane na płyty protez zębowych produkowane jest w wielu odmianach i poddawane jest ciągłym modyfikacjom w celu polepszenia jego właściwości fizyko-mechanicznych [1, 2]. Jednym z istotnych kierunków prac z zakresu protetyki stomatologicznej są badania nad podniesieniem jakości protez wykonywanych z poli(metakrylanu metylu). Protezy akrylowe są stosowane zarówno w leczeniu całkowitego bezzębia, jak i częściowych braków uzębienia. Mimo postępu nadal bardzo często w praktyce klinicznej spotykamy się z uszkodzeniami mechanicznymi płyt akrylowych protez zębowych. Każda poprawa ich trwałości ma znaczenie dla licznej grupy osób, zwłaszcza w wieku starszym [3].

Biorąc pod uwagę powyższe czynniki podjęto badania mające na celu uzyskanie zmodyfikowanego tworzywa akrylowego o lepszych właściwościach wytrzymałościowych. Poprawę właściwości próbowano osiągnąć poprzez wprowadzenie do polimeru napełniacza (bentonitu), czyli wytworzenie kompozytu. Dodatek napełniacza powinien umożliwić wykonanie cieńszej i lżejszej protezy, bardziej komfortowej dla użytkownika. Aby uzyskać ten efekt, napełniacz powinien być maksymalnie rozproszony w matrycy polimerowej.

Kompozyty to materiały uzyskiwane przez połączenie co najmniej dwóch materiałów o różnym charakterze i postaci, posiadających właściwości będące wypadkową właściwości komponentów i ich udziałów objętościowych [4].

Termin "nanokompozyt" opisuje dwufazowy materiał, w którym jedna z faz jest rozproszona w drugiej na poziomie nanometrycznym (10^{-9} m) [5]. Zazwyczaj wymiary struktur

INFLUENCE OF MODIFIED BENTONITEE ADDITION ON ACRYLIC NANOCOMPOSITE PROPERTIES

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Abstract

Modern dental prosthetics is provided with a wide range of materials that may be used for the purpose of stomathognatic system rehabilitation. Frequent damages of prosthesis base structures may however, indicate that acrylic material used to construct the prosthesis bases proves insufficient mechanical strength and resistance characteristics. The article discusses the results of experiments on modification the acrylic material with laminar silicate (bentonitee), aiming at prosthesis structure strength improvement.

Key words: *acrylic nanocomposites - resistance, modified bentonitees*

[Engineering of Biomaterials, 37, (2004), 21-27]

Introduction

Acrylic synthetic material, having been used to make the dental prosthesis bases, is produced in a wide range of variations and subject to continuous modifications to improve its physical-mechanical characteristic [1, 2]. One of the essential research work directions in dental prosthetics field are the researches on improving the quality of dental prosthesis made of poly (methyl-metacrylate). Acrylic dental prosthesis are implemented in treatment of both total toothlessness or partial deficiency of teeth. However, despite technological progress still there are frequent mechanical damages of acrylic dental prosthesis bases encountered in clinical practice. Thus, any and all improvements of acrylic dental prosthesis base durability is significantly important for a large group of patients, in particular elder people [3].

Having the above factors in mind, the research works aiming at providing a modified acrylic material of improved resistance properties have been undertaken. One of the improvement methods is addition of a filler (bentonite) to polymer, i.e. production of a composite. Addition of the filler should enable to produce thinner and lightweight dental prosthesis, thus more comfortable for its user. To achieve the latter effect, a filler should be dispersed in polymer matrix to maximum extent.

Composites are the materials acquired through combination of at least two component materials of different characteristics and form. A composite proves to have the characteristics being the resultant of characteristics of its components and their proportion by volume [4].

The term "nanocomposite" denotes a two-phase material where one phase is dispersed in the other one at nanometric level (10-9 m) [5]. In most cases the size of

obecnych w nanokompozytach zawierają się w przedziale od 1 do 100 nm (rzadziej do kilkuset nm) [6].

Matrycą w nanokompozytach polimerowych mogą być zarówno polimery termoplastyczne jak i termoutwardzalne. Drugim, obok polimeru, składnikiem kompozytów są najczęściej substancje nieorganiczne, przede wszystkim różnego rodzaju krzemiany warstwowe, krzemionka, ale również fulereny, metale i inne związki nieorganiczne [6].

Nanokompozyty polimerowe powstają przez modyfikację tradycyjnych tworzyw. Wprowadzenie do matrycy polimerowej relatywnie małej ilości (kilka części wagowych) składnika rozdrobnionego do rzędu nanometrów (anizotropowe nanocząstki z wysokim współczynnikiem kształtu) w zasadniczy sposób wpływa na osiągnięcie zadanych właściwości [7]. Napełnienie nanokompozytów polimerowych wynosi zazwyczaj 1 do 5 cz. wagowych (maksymalnie 10 cz. wag.).

Po wprowadzeniu nanonapełniacza obserwuje się znaczną poprawę wytrzymałości na rozciąganie i wzrost modułu Younga, wzrasta też odporność na ściskanie [8].

Krzemiany warstwowe, do których należy montmorylonit to najważniejsza grupa nanonapełniaczy. Termin "krzemiany warstwowe" odnosi się do naturalnych minerałów ilastych oraz do syntetycznych krzemianów warstwowych takich jak magadyt, laponit i fluorohektoryt. Montmorylonity stanowią główny składnik skał zwanych bentonitami Zarówno naturalne jak i syntetyczne krzemiany warstwowe są stosowane jako nanonapełniacze w nanokompozytach [8].

Charakterystyczną cechą montmorylonitu jest jego zdolność do sorpcji niektórych kationów i zatrzymywania ich po wymianie, co umożliwia modyfikację tego minerału.

Podstawowe metody otrzymywania nanokompozytów poli(metakrylan metylu) - bentonit można podzielić na dwie grupy. Pierwsza grupa metod polega na polimeryzacji w masie [9] i polimeryzacji w rozpuszczalniku [10], druga obejmuje metody interkalacji w rozpuszczalniku [11] oraz interkalacji w stopie [10].

Reakcję polimeryzacji metakrylanu metylu prowadzi się w obecności zdyspergowanego zmodyfikowanego bentonitu; monomer wnikając między ułożone w pakiet płytki minerału powoduje pęcznienie pakietu aż do separacji płytek. Wzrastające podczas polimeryzacji łańcuchy polimeru zostają zablokowane między warstwami krzemianu, tym samym unieruchamiając montmorylonit i utrwalając separację warstw. W efekcie powstaje kompozyt z napełniaczem o dużym stopniu rozproszenia [10].

Inni autorzy podejmowali próby wzmacniania tworzywa protez poprzez zastosowanie siatek metalowych, tkanin szklanych, nylonowych węglowych i aramidowych Kevlar [12-19].

Przedstawiony artykuł zawiera wstępne wyniki próby modyfikacji tworzywa akrylowego nanocząstkami zmodyfikowanego hydrofobowo bentonitu.

Cel pracy

Określenie wpływu dodatku bentonitu i rodzaju czynnika modyfikującego ten napełniacz na właściwości kompozytu akrylowego.

Materiały i metody

Materiał protetyczny Vertex Rapid Simplifield (Dentimex B.V. Holandia), jest tworzywem na bazie poli(metakrylanu metylu) stosowanym do wykonywania protez zębowych techniką puszkowania. Charakterystykę utwardzonego materiału Vertex R.S. przedstawia TABELA 1.

Vertex Rapid Simplifield został napełniony zmodyfiko-

structures in nanocomposites fall within the range from l up to 100 nm (the cases up to several hundred nm occur rather seldom) [6].

The matrix in polymer nanocomposites can be based on both the thermoplastic and thermally cured polymers. Another composite component, besides the polymer, in most cases are non-organic substances, mainly various types of laminar silicate or silica but also fulerens, metals and other non-organic compounds [6].

Polymer nanocomposites are formed through modification of conventional synthetic materials. Introduction of relatively small amount (several parts by weight) of a component crushed down to nanometer magnitude particle size (anisotropic nanoparticles of high shaping factor) to the matrix significantly influences the required features to be achieved [7]. The polymer nanocomposites get filled up usually from 1 up to 5 parts by weight (max. up to 10 parts by weight).

On having introduced a nanofiller, the composite shows considerable improvement in tensile strength and increase in Young's modulus as well as increase in compressive strength [8].

Laminate silicates, e.g. montmoryllonite, constitute the most important group of nanofillers. The term "laminar silicates" stands for natural clay minerals and synthetic laminar silicates, e.g. magadyt, laponit and fluorohektoryt. Montmoryllonites are the main components of rocks known as bentonites. Both the natural and synthetic laminar silicates are used as nanofillers in nanocomposites [8].

The characteristic feature of montmoryllonite is its sorption capability for certain cations and retaining them after the exchange, thus enabling modification of the mineral concerned.

The methods of producing poly (methyl-metacrylate) nanocomposites - bentonite, can be divided into two groups. The first group of methods is based on polymerization in mass [9] or polymerization in solvent [10], while the other group includes methods of interkalations in solvent [11] and interkalations in alloys [10].

The reaction of methyl-metacrylate polymerization is carried out at presence of dispergated modified bentonite; the monomer penetrating the mineral between mineral plates, composing a pack, causes the pack swelling so as the plates get separated. The polymer chains, expanding during polymerization process, get blocked between the silicate layers, causing the montmoryllonite to get stuck and making the layer separation still. In the result we obtain a composite with a filler, of high dispersion degree [10].
The other authors reported their attempts to strengthen the prosthesis material through using material nets, glass fabric, nylon fabric, carbon fabric or aramide fabric, Kevlar [12-19].

This article presents the first results of acrylic material modification with nanoparticles of hydrophobically modified bentonite.

Research work objective

Determination of effect of bentonite addition and bentonite modifier type on acrylic composite properties.

Materials and methods

The prosthetic material Vertex Rapid Simplifield (Dentimex B.V., Holland) is a synthetic material based on poly (methyl-metacrylate), used to make dental prosthesis using the canning technique. The characteristics of cured Vertex R.S.material has been presented in TABLE 1.

wanymi bentonitami (Organobentonitem Q i Bentonitem AL), których charakterystykę przedstawiono w TABELI 2.

Modyfikowane organofilowo bentonity do materiału protetycznego wprowadzano kilkoma metodami:

- poprzez mechaniczne zmieszanie Organobentonitu Q z polimerem Vertex,
- poprzez rozproszenie Organobentonitu Q w monomerze Vertex na drodze sonifikacji i następne zmieszanie z polimerem Vertex.

Kolejna metoda to polimeryzacja metakrylanu metylu w obecności zmodyfikowanego bentonitu. Następnie produkt polimeryzacji mechanicznie mieszano z polimerem Vertex lub rozpuszczano w monomerze Vertex i dodawano do polimeru Vertex.

Próbki do badań zostały przygotowane poprzez dodanie do odważonej części ciekłej (monomer) określonej ilości części stałej (polimer).

Po szybkim ręcznym wymieszaniu składników mieszanina była nakładana do silikonowej dwuczęściowej formy. Formy były następnie przykrywane folią poliestrową pokrytą środkiem podziałowym i zamykane. Złożona forma była pozostawiona na 1h w temperaturze pokojowej by monomer mógł lepiej zwilżyć i spęcznić polimer. Następnie formę umieszczano w łaźni wodnej w temperaturze 95-98°C i proces polimeryzacji prowadzono przez 2h. Po tym czasie formę wyjmowano z łaźni i pozostawiano do ostygnięcia.

Do analizy właściwości mechanicznych posłużyły dwie metody badań: wytrzymałość na zginanie oraz udarność, najbardziej istotne ze względu na obszar zastosowań badanego materiału. Badanie właściwości mechanicznych wykonane zostało na uniwersalnej maszynie wytrzymałościowej Instron 4026-006 połączonej z komputerem (Instron, USA,1997) według norm PN-EN ISO 527-3 (rozciąganie) i PN-EN ISO 178 (zginanie). Udarność wykonano na młocie Charpy'ego bez karbu według normy PN-81/C-89029 .

Wyniki badań i ich omówienie

Podane wyniki zawierają porównanie właściwości mechanicznych kompozytów w zależności od metody wprowadzenia Organobentonitu Q do układu.

Porównano również wpływ rodzaju bentonitu na właściwości mechaniczne wytworzonego kompozytu, do którego napełniacz dodany został przez sonifikację w monomerze (VOM).

Dla próbek, w których bentonit dodawany był do polimeru (VOP) określono również wpływ udziału napełniacza na właściwości mechaniczne kompozytów.

Wyniki badań mechanicznych podzielone zostały na

Parametr Parameter		Wartość Value
Udarność (bez karbu) Impact resistance (without notch)	[kJ/m^2]	11,3
Wytrzymałość na zginanie Bending strength	[MPa]	85
Moduł sprężystości Young modulus	[MPa]	2367
Zawartość monomeru w gotowej protezie Monomer contents in finished prosthesis	[%]	1,5
*Dane producenta / Producer's data		

TABELA 1. Charakterystyka utwardzonego materiału protetycznego Vertex Rapid Simplified (dane producenta)
TABLE 1. Characteristics of cured prosthetic material Vertex Rapid Simplified (producer's data)

Vertex Rapid Simplifield was filled with modified bentonites (Organobentonite Q or Bentonite AL), the characteristics of which have been presented in TABLE 2.

The organophyllically modified bentonites were added into the prosthetic material using different methods:

- mechanical mixing of Organobentonite Q with polymer Vertex,
- dispersion of Organobentonite Q in monomer Vertex through sonification, followed by mixing the dispersion with polymer Vertex.

Another method was the methylometacrylan polymerization at presence of modified bentonite. The polimerization product was mixed mechanically with polymer Vertex or dissolved in monomer Vertex and added to polymer Vertex.

The testing samples were prepared in the following way: certain amount of solid compound (polymer) was added to certain weighed liquid compound (monomer).

On having the components stirred manually but fast, the mix was put into a two-part silicone mould. The mould was covered with polyester foil, maintaining the separation edge and closed. Then the closed mould was left still at room temperature for 1 hour period to enable the monomer to provide the polymer wetting and swelling better. Next, the mould was placed in water bath at temperature 95-98°C and polymerization process proceeded for 2 hours. After that time period the mould was taken out from the bath and left to get cooled down.

To provide the analysis of mechanical properties there were two methods applied: bending strength and impact resistance, the most important features if concerning the application area of the material tested. Mechanical proper-

Rodzaj bentonitu Bentonit type	Modifier	Charakterystyka Characteristics
Organobentonit Q	pochodna oktadecyloaminy* derivative of oktadecyloaminy*	Proszek koloru kremowego, bez zapachu. Zawartość wody w 80°C– maks. 2%. Pozostałość na sicie o oczku 0,056 mm–maks. 1%. Pęcznienie w mieszaninie ksylen–etanol 98:2 - min. 18 cm^3.* Cream color powder, no odour. Water contents at 80°C– max. 2%. Residue on mesh 0.056 mm – max. 1%. Swelling in the mix ksylen–etanol 98:2 - min. 18 cm^3.*
Bentonit AL	Hyamina 1622 (chlorek diizobutylofenoksyetoksy-etylodimetylobenzyloaminy)**	Źle zwilż alny wodą- nie pęcznieje, pływa na powierzchni wody; pęcznienie w acetonie: 8,5 cm^3/1 g próbki Poor water wetting – no swelling, floats on water surface; swelling in acetone: 8,5 cm^3/1 g of sample
* dane producenta ZGM „Zębiec", Zębiec ** dystrybutor - POCh * Data by the producer, ZGM „Zębiec", Zębiec ** Distributor - POCh		

TABELA 2. Charakterystyka zmodyfikowanych hydrofobowo bentonitów.
TABLE 2. Characteristics of hydrophobically modified bentonites.

dwie serie, seria druga wykonana została dla 1% wag. zawartości napełniacza. Na wykresach zaznaczone zostały odchylenia standardowe.

W pierwszej serii badań określony został wpływ metody wprowadzania bentonitu do materiału protetycznego:

- dodatek do monomeru Vertrex (VOM-1%),
- dodatek do polimeru Vertex (VOP-0 -5%).

Z danych zamieszczonych na RYS.1 wynika, że wytrzymałość na zginanie materiału protetycznego Vertex z dodatkiem napełniacza wyraźnie spada. Nawet niewielki dodatek bentonitu (VOP-0,2%) powoduje obniżenie wytrzymałości na zginanie o ok. 18%. Wartość 77,7 MPa dla czystego materiału (VOP-0%) jest niższa od minimalnej podawanej przez producenta Vertexu (85 MPa).

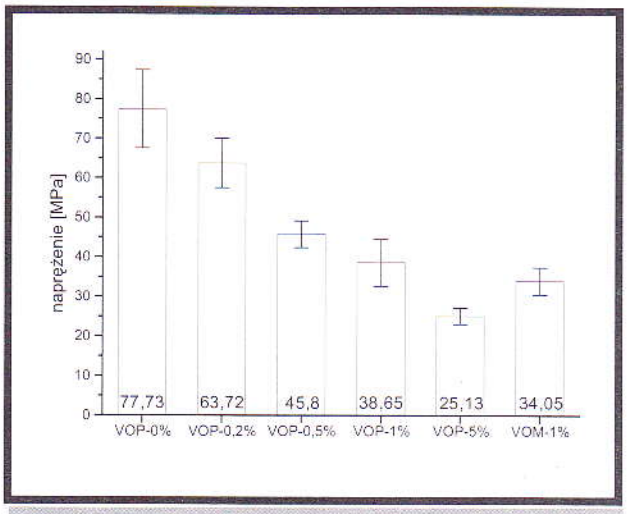
W przypadku modułu Younga (RYS.2) obserwuje się pewien wzrost jego wartości w porównaniu do czystego materiału (VOP-0%; 2220 MPa), dla kompozytów VOP-0,2% (2602 MPa) i VOP-5% (2580 MPa) oraz porównywalną wielkość z nienapełnionym polimerem dla kompozytu VOP-0,5% (2217 MPa). Ze wzrostem udziału napełniacza następuje spadek wartości strzałki ugięcia (spadek elastyczności kompozytu). Brak także wyraźnego wpływu sonifikacji na poprawę wartości modułu Younga. Minimalny moduł Younga podany przez producenta dla materiału Vertex wynosi 2367 MPa, a więc jest wyższy niż oznaczony przez nas dla niemodyfikowanej próbki tworzywa akrylowego.

Na RYS. 3 zestawiono wyniki pomiarów udarności kompozytów Vertex - bentonit. Widoczny jest znaczny spadek wytrzymałości udarnościowej badanych kompozytów, ponad dwukrotny przy 0,5% udziale napełniacza. Kompozyt otrzymany przez rozproszenie bentonitu w monomerze Vertex (VOM-1%) cechuje lepsza charakterystyka udarnościowa niż odpowiadający mu kompozyt VOP-1% (napełniacz wymieszany mechanicznie z polimerem).

W drugiej serii badań bentonit (Organobentonit Q) został rozproszony w poli(metakrylanie metylu) w trakcie polimeryzacji. Następnie określono wpływ sposobu wprowadzenia bentonitu interkalowanego PMMA do materiału protetycznego:

- przez rozpuszczenie w monomerze Vertex (VPM),
- przez zmieszanie z polimerem Vertex (VPP) (zdjęcie) .

Wykonano i przebadano również kompozyt(VALM), w którym hydrofobowy Bentonit AL rozproszono przez sonifi-



RYS. 1. Wpływ udziału napełniacza na wytrzymałość na zginanie kompozytów pierwszej serii.
FIG. 1. Influence of filler fraction on composite bending strength for the first series composites.

ties of the material were tested by means of a versatile resistance testing machine Instron 4026-006 linked to the computer (Instron, USA,1997), in accordance to Polish standards PN-EN ISO 527-3 (tensile) and PN-EN ISO 178 (bending). The material impact resistance was tested on Charpy's impact machine without the notch, in accordance to Polish standard PN-81/C-89029.

Test results and discussion

To present the test results, the comparison of composite mechanical properties was done in relation to the method of introducing the Organobentonite Q to the system.

Another comparison concerned the influence of bentonite type on mechanical properties of the composite where the filler was added in the process of sonification in monomer (VOM).

For the samples where bentonite was added to the polymer (VOP), the influence of filler portion on composite mechanical properties was also determined.

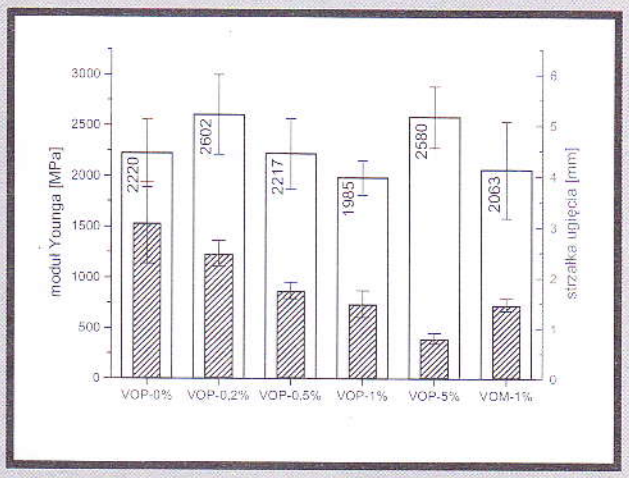
The results of mechanical examination were achieved in two series of tests, where the second series was carried out for filler contents equal to 1% by weight. The curves on the charts presented below have the standard deviation values marked thereon.

The first series of tests was used to determine the influence of method if introducing bentonite to prosthetic material:

- adding the bentonite to monomer Vertrex (VOM-1%),
- adding the bentonite to polymer Vertex (VOP-0 -5%).

The chart in FIG.1 confirms an apparent decrease in bending strength of prosthetic material Vertex with addition of the filler. Even a small fraction of bentonite (VOP-0,2%) causes the decrease in bending strength by about 18%. The value 77.7 MPa for the pure material (VOP-0%) is lower than the minimum value indicated by the producer of Vertex (85 MPa).

The values of Young's modulus (FIG. 2) show value increase when compared to pure material (VOP-0%; 2220 MPa), for the composites VOP-0.2% (2602 MPa) and VOP-5% (2580 MPa), and comparable value to non-filled polymer value for the composite VOP-0.5% (2217 MPa). Along with the increase in filler fraction the value of deflection gradient decreases (drop of composite flexibility). Moreover, no apparent influence of sonification on Young's modulus



RYS. 2. Moduł Younga oraz strzałka ugięcia podczas zginania kompozytów pierwszej serii.
FIG. 2. Young's modulus and deflection gradient during bending the first series composites.

kację w monomerze. Wszystkie kompozyty drugiej serii zawierały 1% wag. napełniacza.

Na RYS. 4 widać przywarte do powierzchni cząstek poli(metakrylanu metylu) mniejsze cząstki polimeru Vertex; przyciąganie na zasadzie sił elektrostatycznych jest trwałe dzięki rozwiniętej powierzchni kontaktu cząstek.

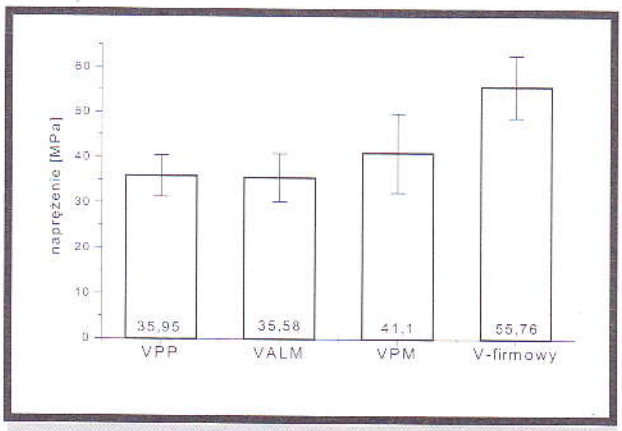
Z danych zamieszczonych na RYS. 5 wynika, że - podobnie jak w pierwszej serii - właściwości mechaniczne materiału protetycznego z dodatkiem napełniacza uległy pogorszeniu. Z przebadanych kompozytów największą wytrzymałość na zginanie ma kompozyt VPM (41,1 MPa).

Z RYS. 6 wynika, że moduł Younga wzrasta dla wszystkich modyfikowanych materiałów protetycznych. Największy wzrost w porównaniu do nienapełnionego tworzywa (V-firmowy, 1768 MPa) obserwuje się w przypadku kompozytów VPP (2289 MPa, wzrost o 30%) i VPM (2243 MPa wzrost o 27%). (Różnice w oznaczonych wartościach wytrzymałości dla próbek materiału nienapełnionego otrzymanych w pierwszej i drugiej serii wynikają z różnego sposobu ich przygotowania oraz trudności z odpowietrzeniem kompozycji). Spadają wartości strzałek ugięcia modyfikowanych materiałów; największą elastyczność spośród napełnionych kształtek posiada kompozyt VPM.

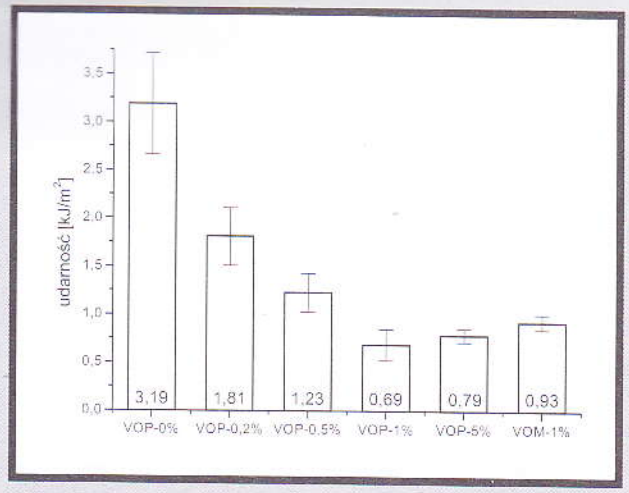
Wartość udarności materiału nienapełnionego (V-0) wynosi 2,1 kJ/m^2; kompozyt VPM ma udarność najbardziej do niej zbliżoną (1,66 k J/m^2) (RYS. 7).

Porównując próbki kompozytów z dwoma różnymi modyfikowanymi hydrofobowo bentonitami, wprowadzonymi poprzez sonifikację (Organobentonit Q - VOM-1% i Bentonit AL - VALM) nie stwierdzono istotnej różnicy w wytrzymałości na zginanie.

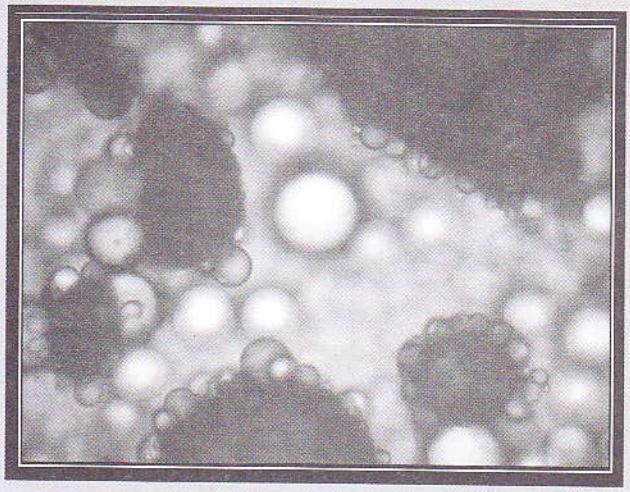
Przedstawione powyżej metody praktycznie wyczerpują metody modyfikacji materiału protetycznego bentonitem bez ingerencji w strukturę polimeru Vertex. W przypadku próbek, w których bentonit dodawany był do polimeru (seria VOP) na pogorszenie właściwości kompozytów miał prawdopodobnie wpływ krótki czas kontaktu napełniacza z monomerem (konkurencyjny jest tu proces spęczniania polimeru przez monomer). Niższe wartości właściwości wytrzymałościowych dla nienapełnionych materiałów i różnice w tych wartościach w porównaniu z danymi producenta mogły być związane trudnościami w uzyskaniu znormalizowanych kształtek do badań, przy jednoczesnym zachowaniu warunków zbliżonych do wykonywania protez akrylowych.



RYS. 5. Wpływ sposobu wprowadzania i rodzaju czynnika modyfikującego na wytrzymałość na zginanie kompozytów drugiej serii.
FIG. 5. Influence of modifier introduction method and modifier type on bending strength of the second series composites.



RYS. 3. Wytrzymałość udarnościowa kompozytów pierwszej serii [kJ/m^2].
FIG. 3. Impact resistance of the first series composites, [kJ/m^2].

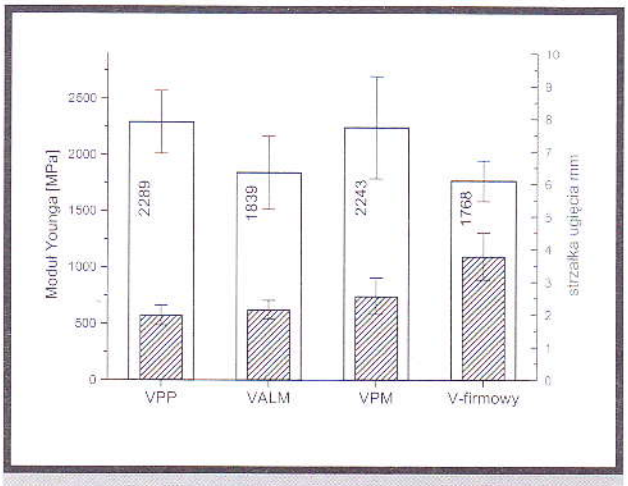


RYS. 4. Zdjęcie mikroskopowe mieszaniny Vertexu i poli(metakrylanu metylu) zawierającego bentonit.
FIG. 4. Microscope photo of the mix of Vertex and poly(methyl-metacrylate) containing bentonite.

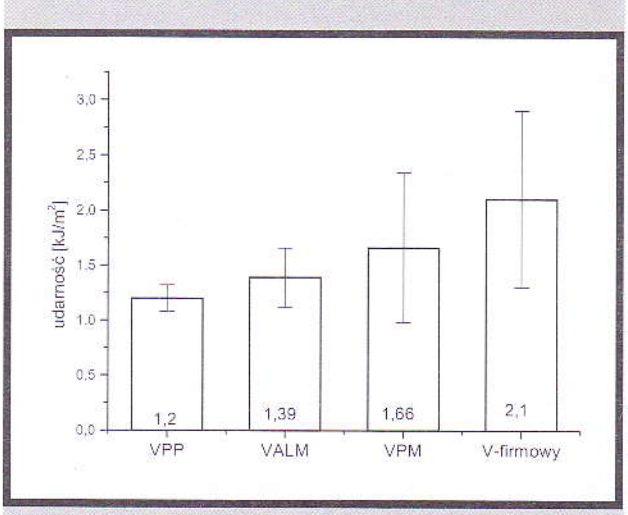
value improvement can be noticed. The minimum value of Young's modulus for Vertex material, according to producer data, is 2367 MPa. The latter value is higher than the value determined in the course of our tests on non-modified acrylic material sample.

FIGURE 3 presents the impact resistance measurement results for the composites of Vertex with bentonite filler. The chart shows a significant decrease in impact resistance value of the composites tested; the filler contents of 0.5% causes the impact resistance value to decrease over two times down. The composite obtained through bentonite dispersion in monomer Vertex (VOM-1%) shows better impact characteristics than the corresponding composite VOP-1% (the filler mechanically mixed with polymer).

The other series of tests concerned the composites where bentonite (Organobentonite Q) was dispersed in poly (methyl-metacrylate) during polymerization. The tests allowed to determine the influence of method of introducing interkalowanego bentonite PMMA to prosthetic material. The methods included:



RYS. 6. Moduł Younga oraz strzałka ugięcia podczas zginania kompozytów drugiej serii.
FIG. 6. Young's modulus and deflection gradient during bending the second series composites.



RYS. 7. Wpływ sposobu wprowadzania i rodzaju czynnika modyfikującego na wytrzymałość udarnościową kompozytów drugiej serii [kJ/m²].
FIG. 7. Influence of modifier introduction method and modifier type on second series composite impact resistance [kJ/m²].

Wnioski

1. Wstępne próby uzyskania materiału protetycznego o podwyższonych właściwościach mechanicznych nie zakończyły się powodzeniem.
2. Przedstawione wyniki badań podczas zginania oraz badań udarnościowych pozwalają na stwierdzenie, ze dodatek napełniacza w jakiejkolwiek z przedstawionych postaci pogarsza właściwości mechaniczne tworzywa akrylowego.
3. Na polepszenie wytrzymałości powinno w znaczący sposób wpłynąć zastosowanie bentonitu o większej zawartości montmorylonitu, do modyfikacji którego użyto związek zawierający wiązanie podwójne, co umożliwi chemiczne związanie napełniacza z matrycą polimerową.

PRZYCZYNY

- bentonite dissolution in monomer Vertex (VPM),
- bentionit mixing with polymer Vertex (VPP) (see the photo below).

Additionally, there was another composite prepared and tested, i.e. composite (VALM) where hydrophobic Bentonite AL was dispersed in monomer through sonification process. There was 1% of filler, by weight, in each second series composite.

The photo in FIG. 4 reveals the particles of Vertex polymer (smaller ones) attached to the surface of poly (methylmetacrylate) particles; the attachment, based on electrostatic forces, remains durable due to the particle contacting spanned surface.

The data indicated in FIG. 5 confirm that - similarly as in case of first series composites - the properties of prosthetic material with filler contents got worsened.

From among the composites tested, the composite VPM (41,1 MPa) proved to have the best bending strength value.

On base of FIG. 6 it can be stated that Young's modulus increases for each modified prosthetic material. The highest increase, in comparison to material without any filler added (V-0, 1768 MPa), can be noticed in case of composites VPP (2289 MPa, increase by 30%) and VPM (2243 MPa, increase by 27%). (The differences in resistance values determined for non-filled material samples obtained for the first and second series of composites result from difference in methods used for composite preparation and difficulties in composite deaeration). The deflection gradients of modified materials show decrease in values; from among the filler containing materials the VPM-composite shows the highest flexibility.

The impact resistance value of non-filled material (V-0) is 2,1 kJ/m^2; the impact resistance value of VPM-composite (1,66 k J/m^2) appears to be the closest value to that of V?0 material (FIG. 7).

On having compared the samples of composites filled with two different bentonites modified hydrophobically, added through sonification (Organobentonite Q- VOM-1% and Bentonite AL-VALM), no significant difference in bending strength values has been observed.

The experimental methods presented herein are in fact the only methods enabling the prosthetic material to be modified with bentonite without interfering in Vertex polymer structure. In samples where bentonite was added to polymer (VOP series), the composite properties proved to get worse probably due to short time of filler's contact with monomer (in that case the process of polymer swelling with monomer appears to prevail). The lower values of resistance parameter values in case of filler-free materials and differences in those values, comparing to producer data, could result from difficulties in producing standard shape samples at simultaneous upholding the conditions possibly the closest to the conditions of acrylic prosthesis construction.

Conclusions

1. The attempts to achieve the prosthetic material of improved mechanical properties proved to have been unsuccessful.
2. The test results concerning bending strength and impact resistance allow to conclude that filler addition, regardless the filler type or methods, makes the acrylic material mechanical properties to worsen.
3. An improvement in resistance and strength parameters should be expected in case the bentonite of higher contents of montmorylonite is added, where bentonite would be modified with a double bond compound, thus enabling chemical binding of filler with polymer matrix.

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NIEPOWODZEŃ W LECZENIU IMPLANTOLOGICZNYM

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Słowa kluczowe: *planowanie leczenia protetycznego implanty zębowe*

[Inżynieria Biomateriałów, 37, (2004), 27-29]

Implantoprotetyka stała się już uznaną metodą postępowania leczniczego, zwłaszcza u pacjentów, u których warunki anatomiczno-fizjologiczne w jamie ustnej są trudne a tradycyjne metody leczenia nie dają dobrych rezultatów. W sytuacjach tych, leczenie protetyczne oparte na implantach wydaje się być leczeniem z wyboru. Należy jednak zdawać sobie sprawę z ryzyka związanego z leczeniem, które wynika ze złej oceny warunków klinicznych i nieprawidłowego zaplanowania uzupełnień protetycznych. W planowaniu leczenia, poza wnikliwą oceną istniejących warunków oraz stanu ogólnego pacjenta należy uwzględnić jego oczekiwania oraz możliwości ich spełnienia [9,14,15,16,17]. Tak więc sukces w leczeniu implantologicznym zależy od:

- właściwego doboru pacjenta,
- prawidłowego planowania,
- dobrej osteointegracji,
- prawidłowego obciążenia,
- użycia materiałów o wysokiej odporności mechanicznej (statycznej i zmęczeniowej)
- dobrej higieny i stałego kontaktu pacjenta z lekarzem,

THE CAUSES OF UNSUCCESSFUL IMPLANTOLOGIC TREATMENT RESULTS

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Key words: *planning of prosthetic treatment, dental implants*

[Engineering of Biomaterials, 37, (2004), 27-29]

Implantoprosthetic has already become an approved dental treatment method, particularly in patients whose oral cavity has displayed difficult anatomic-physiological conditions, where conventional treatment methods do not provide satisfactory results. In such cases the implant based prosthetic treatment seems the appropriate treatment choice. However, we should be aware of risks connected with the treatment undertaken in a result of inappropriate evaluation of clinical conditions and incorrect planning of prosthetic restoration elements. While planning the prosthetic treatment, besides the thorough evaluation of existing conditions and general patient's state, the patient's expectations and possibilities to meet those expectations should be considered [9,14,15,16,17]. Therefore, the implantologic treatment success depends on:

- relevant patient selection,
- correct planning,

• osiągniętej estetyki.

Do leczenia protetycznego zgłaszają się pacjenci zarówno doświadczeni pozytywnie lub negatywnie, jak i niedoświadczeni protetycznie. Bardzo często, to, czego pacjent spodziewa się po leczeniu implantologicznym jest nierealne w stosunku do możliwości wynikających z sytuacji wewnątrzustnej. Zdarza się też, że pacjent oczekuje, iż poprawa wyglądu usunie głęboko tkwiące w nim zaburzenie o podłożu psychicznym. Z takimi pacjentami, źle zdiagnozowanymi są największe problemy. Często wynikają one, nie z niewłaściwie przeprowadzonego leczenia, ale z nierealnych oczekiwań pacjenta.

Większość niepowodzeń wynika ze złego doboru pacjenta, niekorzystnych warunków miejscowych, nieprawidłowego wprowadzenia implantów, braku osteointegracji, złych właściwości materiału użytego do rekonstrukcji. Ewentualna utrata wszczepu w fazie późniejszej może wynikać z niewłaściwego obciążenia konstrukcją protetyczną, braku odległej opieki nad pacjentem oraz wynikającej z tego złej higieny. Należy również zdawać sobie sprawę z tego, że pacjent zgłaszający się do leczenia musi wiedzieć jakie ma możliwości alternatywnego leczenia protetycznego i to on podejmuje decyzję. Ryzyko niepowodzenia spoczywa zarówno na lekarzu jak i pacjencie [12,18].

Zła ocena warunków protetycznych i nie rozpatrywanie narządu żucia jako całości, nieuwzględnienie współistniejącego bruksizmu lub wzmożonej aktywności parafunkcjonalnej, a także zła higiena jamy ustnej i szybko postępujące periodontopatie w efekcie wpływają na ostateczny wynik leczenia. Przedimplantologiczna analiza modeli i zdjęć rtg ze znacznikami pozwala na wstępne określenie liczby oraz umiejscowienia implantów. Badanie grubości błony śluzowej na wyrostku zębodołowym umożliwia przybliżone ustalenie położenia powierzchni nośnej implantu.

Aby właściwie leczyć trzeba posiadać rozległą wiedzę dotyczącą układu stomatognatycznego, ponieważ terapia stomatologiczno-protetyczna to pełna rehabilitacja narządu żucia w odniesieniu do wszystkich jego funkcji. W warunkach uzębienia naturalnego siły żucia przenoszone są na kości szczęki i żuchwy poprzez zęby. Siła nacisku amortyzowana jest przez aparat zawieszeniowy zębów a wielkość sił żucia regulowana jest poprzez proprioreceptory znajdujące się w ozębnej. Również zęby zróżnicowane są morfologicznie i dzięki temu bardziej dostosowane do zmiennych wielkości sił obciążeniowych w poszczególnych obszarach narządu żucia. Proces wgajania implantu polega na osteointegracji, a połączenie pomiędzy wszczepem i kością jest połączeniem sztywnym. W związku z tym siły przenoszone przez zrekonstruowane na implantach uzębienie nie są amortyzowane tak jak ma to miejsce w uzębieniu naturalnym. Różnorodna struktura kości szczęk i żuchwy oraz "nieprzewidywalne" obciążenia z jakimi mamy do czynienia w jamie ustnej są jednym z czynników wpływających na niepowodzenie leczenia.

Stosunkowo częstą przyczyną problemów w leczeniu protetycznym jest nieprawidłowe wprowadzenie implantów [3]. Dotyczy to zarówno implantów wprowadzonych nieosiowo lub w zbyt małej liczbie, jak również złego ich wymiaru (długość, średnica) oraz złej lokalizacji bez uwzględnienia planowania protetycznego oraz biomechaniki przenoszenia obciążeń okluzyjnych [5, 6, 7, 8, 10, 11, 13, 21, 22].

Późne niepowodzenia w leczeniu implantologicznym wynikają najczęściej z nieprawidłowo wykonanej nadbudowy protetycznej. Zazwyczaj jest to:

- zbyt rozległa powierzchnia okluzyjna,
- zbyt aktywne kontakty okluzyjne,
- niecentryczne kontakty zwarciowe
- zbyt rozległa konstrukcja na małej liczbie implantów,

- good osteointegration,
- appropriate structure load arrangement,
- applying materials of high mechanical resistance (static and fatigue resistance)
- good hygiene and continuous contact between the patient and dentist,
- esthetic level achieved.

Among the patients to undergo the prosthetic treatment there are patients with both positive or negative prosthetic experiences, as well as the inexperienced at all. In fairly many cases the patient's expectations, concerning the implantologic treatment, appear unreal against actual possibilities arising from intra-cavity conditions. Sometimes a patient happens to expect that his/her teeth appearance improvement should remove certain disorders developed due to deep psychical factors . Such patients, having been improperly diagnosed at initial implantologic treatment phase, would cause the most problems. Quite frequently the problems result not from the improper treatment but unreal patient's expectations.

Most failures results from irrelevant patient selection, adverse oral cavity conditions, improper implant introduction, lack of osteointegration or poor properties of materials used for reconstruction. Possible loss of implant in later phase can be the result of inappropriate load with prosthetic structure, lack of further care over the patient and thus poor hygiene resulting thereof. Moreover, it should be pointed out that a patient applying for the prosthetic treatment must be informed about possible prosthetic treatment alternatives and it is the patient who actually makes the decision. The risk of potential failure rests on both the dentist and patient [12, 18].

Incorrect evaluation of prosthetic conditions and failure to concern the masticatory organ as an entire system, disregarding the co-existing bruxism or parafunctional overactivity as well as poor hygiene of oral cavity and rapidly progressing periodontopathy, would affect the final results of the treatment. The pre-implantologic analysis of models and X-ray pictures with indices enable preliminary determination of implants quantity and location. The examination of mucous membrane thickness on alveolar appendix should enable estimation of implant carrying surface position.

The proper treatment requires extensive knowledge on stomatognathic system since the dental-prosthetic therapy means full rehabilitation of masticatory organ concerning all its functions. In case of natural teeth the masticatory forces are passed on to the jaw bones and mandibula bones with the teeth. The impact force is amortized with teeth suspension apparatus while the mastication force level id controlled by the proprioreceptors located in periodontium. Moreover, the teeth are diversified in terms of their morphology and thus individual teeth are adapted to varying load forces in particular areas of masticatory organ. The process of implant cicatrization involves osteointegration and the joint between the implant and the bone is a rigid joint. Therefore, the forces carried by the teeth reconstructed with implants lack of the amortization which occurs in case of natural teeth. The diversified structure of jaw bones and mandibula bones as well as "unpredictable" loads found in oral cavity, are among the factors leading to treatment failures.

The relatively frequent cause of problems in prosthetic treatment is the improper installation of implants [3]. The latter covers such disorders as non-axial installation or insufficient number of implants or inaccurate implant size (length, diameter) or inappropriate implant placement with no regard to prosthetic planning or occlusion load transfer biomechanics [5, 6, 7, 8, 10, 11, 13, 21, 22].

- niekorzystny stosunek długości implantu do korony
- nieszczelności pomiędzy implantem a nadbudową protetyczną
- wady materiałowe wszczepów i konstrukcji protetycznych.

Czasami lekarz planując leczenie źle ocenia dane estetyczne, a więc rozmieszczenie zarysu dziąsła, brodawek międzyzębowych, przebieg linii śmiechu czy wysokość bezzębnych odcinków w stosunku do pozostałych zębów. Wyżej wymienione elementy mogą być przyczyną późniejszych problemów z uzyskaniem dobrego efektu estetycznego przez lekarza protetyka i zdarza się, że kończy się to reoperacją.

Podsumowanie

Prawidłowe planowanie leczenia implantologicznego zmniejsza ryzyko niepowodzenia w leczeniu, których nie jest się w stanie przewidzieć do końca i mogą się one zdarzyć zarówno w sytuacjach wydawałoby się "łatwych" jak i "trudnych". Dlatego też sukces leczenia implantologicznego zależy od ścisłej współpracy pomiędzy chirurgiem, lekarzem protetykiem, technikiem dentystycznym, mimo że odpowiedzialność i opieka długoterminowa spoczywa na lekarzu protetyku. Szeroko pojęta współpraca z inżynierami, którzy konstruują implanty w oparciu o szczegółowe dane biomechaniczne właściwości kości szczęk powinna zaowocować opracowaniem doskonalszych metod rehabilitacji narządu żucia. Wydaje się jednak, że przyszłość leży prawdopodobnie w inżynierii tkankowej.

The subsequent failures of implantologic treatment the most often result from improperly made prosthetic overstructure. In most cases it concerns the following:

- too widespread occlusion surface;
- too active occlusion contacts;
- non-centered occlusion contacts;
- too excessive structure over a few implants,
- unfavorable ratio between the implant length and the crown
- lack of tightness between the implant and the prosthetic overstructure;
- defects in implant material or prosthetic structure material

In the course of treatment planning a dentist may evaluate inappropriately the esthetic elements, e.g. gum contour outline, inter-tooth warts, shape of smile line or the height of teeth-free segments in reference to adjacent teeth. The elements mentioned can cause further problems in achieving relevant esthetic effect by prosthetic surgeon, and it happens the reoperation is necessary.

Summary

Appropriate implantologic treatment planning allows to reduce the risks of treatment failures which are difficult to predict and may unexpectedly occur in circumstances evaluated as seemingly "easy" and those recognized as "difficult". Therefore, the final success of implantologic treatment depends on strict cooperation between the prosthetic surgeon, prosthetic dentist and prosthetic technician, even though the overall responsibility and long-term care rests on the prosthetic dentist. The cooperation, in its wide meaning, with engineers constructing the implants, on base of detailed biomechanical data on jaw bone characteristics, should give rise to development of increasingly improved methods of masticatory organ restoration. It seems, however, the future prospective potential should come out along with tissue engineering advancement and innovations.

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WŁASNA METODA KONSERWACJI PRZESZCZEPIANYCH NARZĄDÓW MIĄŻSZOWYCH Z ZASTOSOWANIEM POLIETYLENOWEGO ZBIORNIKA

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Streszczenie

W pracy przedstawiono metodę eliminacji czasu ciepłego niedokrwienia przez zastosowanie specjalnie skonstruowanego woreczka polietylenowego. Woreczek zbudowany jest z folii polietylenowej HDPE o niskiej gęstości wytwarzanej pod wysokim ciśnieniem. Własna konstrukcja woreczka (trzy przestrzenie i polietylen) umożliwia przetrzymywanie przeszczepianego narządu w stałej temperaturze +4 stopni Celsjusza. Dzięki zastosowanej metodzie eliminacji czasu ciepłego niedokrwienia można spodziewać się lepszej wczesnej funkcji organu po przeszczepie.

Słowa kluczowe: *czas ciepłego niedokrwienia, przeszczep narządu, zbiornik polietylenowy*

[Inżynieria Biomateriałów, 37, (2004), 30-33]

Wstęp

Czas ciepłego niedokrwienia dzieli się na pierwszy czas ciepłego niedokrwienia obejmujący okres od zatrzymania krążenia dawcy do schłodzenia pobieranych narządów płynem perfuzyjnym oraz czas drugiego ciepłego niedokrwienia [1-7]. Czas ten mierzony od wyciągnięcia narządu z lodu do czasu odtworzenia przepływu krwi przez naczynia i jest zawsze związany ze wzrostem temperatury przeszczepianego i jednocześnie niedokrwionego narządu [1-3]. Niedokrwienie i wzrost temperatury są czynnikami uszkadzającymi przeszczepiany narząd, co w konsekwencji może prowadzić do jego opóźnionej funkcji [3, 4, 8].

Opis metody

W Klinice opracowano własną metodę eliminacji czasu drugiego ciepłego niedokrwienia przez zastosowanie specjalnie skonstruowanego polietylenowego woreczka, w którym umieszczany jest przeszczepiany narząd (np. nerka) w czasie od wyciągnięcia z lodu do odtworzenia przepływu naczyniowego. Woreczek zbudowany jest z folii polietylenowej (-[CH_2-CH2-]$_n$-) o niskiej gęstości wytwarzanej pod wysokim ciśnieniem (HDPE - High Density Polyethylene). Zastosowano folię polietylenową o grubości 0,04 mm. Folię cięto w odpowiednie formy a następnie łączono przy użyciu specjalnej zgrzewarki typu SMS-350 (Super Magnet Se-

THE OWN METHOD OF PRESERVATION OF TRANSPLANTED PARENCHYMAL ORGAN WITH THE USAGE OF POLYETHYLENE RECEPTACLE

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Summary

In this study a method of elimination of the warm ischemia is shown. The method is based on the application of a specially constructed polyethylene bag. The bag is built of polyethylene foil HDPE of low density produced under high pressure. Own construction of the bag (three spaces and polyethylene) enables the storage of a transplanted organ at the stable temperature +4 Celsius degrees. Due to the applied method of the elimination of the warm ischemia one can expect better early organ function after transplantation.

Key words: *warm ischemia time, organ transplantation, polyethylene receptacle*

[Engineering of Biomaterials, 37, (2004), 30-33]

Introduction

The time of warm ischemia can be divided into the first time of warm ischemia comprising the period from the circulatory arrest of a donor to the cooling of the harvested organs with perfusion fluid, and the time of the warm ischemia. The latter is measured from the replacement of the organ from ice to the time of the reconstruction of blood flow through organ vessels, and is always associated with the temperature rise of the transplanted and ischemic organ. Ischemia and temperature rise are factors damaging the transplanted organ, which as a result can lead to its delayed function [3, 4, 8].

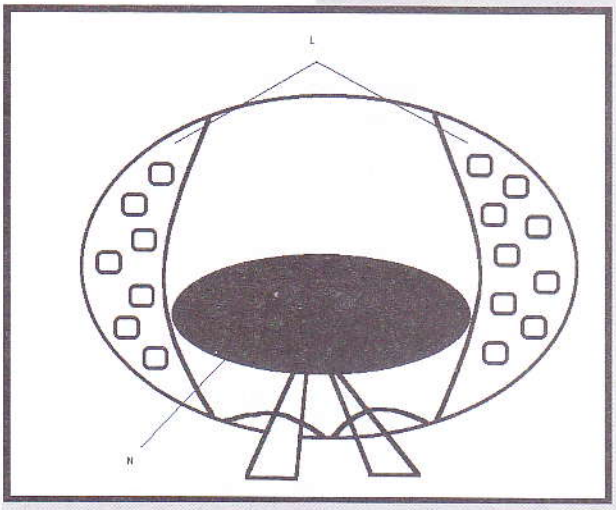
Method description

In our clinic we create the own method of time elimination of the warm ischemia through application of a specially constructed polyethylene bag, in which the transplanted organ (fe. kidney) is placed from the time of its removal from ice to the reconstruction of vessel flow. The bag is built of polyethylene foil (-[CH_2-CH_2-]$_n$-) of low density produced under high pressure (HDPE - High Density Polyethylene). The applied foil was 0,04 mm thick. It was cut in appropriate forms and connected using a special sealer SMS-350 (Super Magnet Sealer). As a result a polyethylene receptacle consisting of three compartments was formed. The bags were sterilized with the use of ethylene oxide, taking advantage of low temperature of this gas

aler). Wytwarzano w ten sposób zbiornik polietylenowy zbudowany z trzech kompartmentów. Woreczki sterylizowano za pomocą tlenku etylenu wykorzystując niską temperaturę działania tego gazu, jego zdolności przenikania i wnikania w głąb sterylizowanego tworzywa oraz zjawisko adsorpcji. Sterylizacja trwa 6 godzin. Wysterylizowane zbiorniki polietylenowe poddawano degazacji w temperaturze pokojowej przez 7 dni. Konstrukcja woreczka umożliwia przetrzymywanie przeszczepianego narządu w stałej temperaturze +4 stopni Celsjusza dzięki możliwości otoczenia narządu roztworem soli fizjologicznej z topniejącym lodem. Worek do przechowywania nerki musi spełniać warunek schłodzenia nerki do określonej temperatury i oddzielenia przeszczepianego narządu od topniejącego lodu oraz swobodne wykonywanie zespoleń naczyniowych. Realizacja tych założeń była możliwa dzięki wydzieleniu w pojemniku trzech przestrzeni: dwóch służących jako zbiorniki na topniejący lód otaczających trzeci, bez płynu, przeznaczony do przechowywania nerki na czas zespoleń naczyniowych (RYS.1). Wydzielenie zbiornika na nerkę umożliwiało wygodny dostęp do naczyń nerkowych (tętnicy i żyły) dzięki wycięciu dwóch otworów w podstawie tego zbiornika. Dodatkowo górne bieguny naczyń przeszczepianej nerki były zaznaczane szwami naczyniowymi w celu uniknięcia ich skręcania w czasie wykonywania zespoleń. Zbiorniki na topniejący lód pozostawały w tym czasie nienaruszone i dzięki temu mogły spełniać rolę rezerwuaru chłodzącego nerkę. Po odtworzeniu przepływu krwi przez przeszczepianą nerkę worek z topniejącym lodem usuwano umożliwiając wykonanie dalszych etapów transplantacji.

Omówienie

Najlepszym sposobem zabezpieczenia narządów przed uszkodzeniami powstającymi w czasie ciepłego niedokrwienia jest obniżenie temperatury do 4 stopni Celsjusza, w której zwolnieniu ulega proces metabolizmu tlenowego, a zapotrzebowanie na tlen spada do 5% w porównaniu do zapotrzebowania w temperaturze 37 stopni Celsjusza [3, 4,8]. Czas ciepłego niedokrwienia obejmuje w praktyce tylko czas drugiego ciepłego niedokrwienia. Regułą w procedurze pobierania narządów a w tym także nerek jest pozyskiwanie organów do przeszczepu u dawców z zachowaną akcją serca [9]. W tej sytuacji pierwszy czas ciepłego niedokrwienia nerek jest równy zero, ponieważ zimną perfuzję rozpoczyna się jeszcze w czasie pracy serca [9]. Eliminacja czasu ciepłego niedokrwienia była możliwa przez umieszczanie przeszczepianej nerki w woreczku polietylenowym HDPE własnej konstrukcji [10]. Dzięki zastosowaniu polietylenu HDPE o niskiej gęstości wytwarzany zbiornik odznacza się dużą miękkością, co ułatwia przechowywanie nerki w czasie transplantacji. W konstruowaniu worka wykorzystano odporność mechaniczną polietylenu oraz jego odporność na działanie czynników chemicznych i niskich temperatur. Dzięki wydzieleniu zbiorników na



RYS.1. Schemat worka polietylenowego z topniejącym lodem i narządem (N - zbiornik z narządem, L - zbiorniki z topniejącym lodem) - przekrój poprzeczny.
FIG.1. Scheme of polyethylene receptacle with melting ice and with organ (N - the container with organ, L - two container with melting ice) - cross section.

functioning, as well as its capability of penetrating into sterilized material, and adsorption phenomenon. Sterilization lasted 6 hours. The sterilized polyethylene receptacles underwent degassing at room temperature for 7 days. The bag construction allows for the storage of a transplanted organ at a stable temperature +4 Celsius degrees due to opportunity of surrounding the organ with an isotonic salt solution with melting ice. The bag for kidney storing has to meet the requirements of cooling a kidney to the defined temperature and separating the transplanted organ from the melting ice, as well as an unrestrained performance of the vessel anastomosis. Realization of these assumptions was possible thanks to separating three spaces in the container: two serving as reservoirs for melting ice, surrounding the third, without liquid, assigned for storing the kidney during the time of vessel anastomosis (FIG.1). Separating a special renal container enabled convenient access to renal vessels (an artery and a vein) thanks to a cutting of two openings at the basis of this container. Additionally, upper poles of the vessels of the transplanted kidney were marked by vascular sutures to avoid their twisting during performance of the anastomosis. At the same time the reservoirs for melting ice remained untouched, which enabled them to exert a cooling function for the kidney. After the reconstruction of the blood flow through the transplanted kidney, the bag with the melting ice was removed, thus allowing for the performance of the further stages of transplantation.

Discussion

The best way of protection of organs against deleterious effects of warm ischemia is lowering the temperature to 4 Celsius degrees. In such conditions oxygen metabolism slows down and oxygen demand decreases to 5% in comparison to the requirement at the temperature 37 Celsius degrees [3, 4, 8]. Therefore, the time of warm ischemia comprises practically only the time of the second warm ischemia. However, the rule in organ harvesting procedure is taking organs for transplantation from donors with heart action [9]. In such a situation the first time of warm renal ischemia is reduced to zero, because cold perfusion starts yet during heart work [9]. Elimination of the time of warm ischemia was possible through the placement of the transplanted kidney in the polyethylene bag of our own construction [10]. Kidney transplantation was possible also through the usage soft polyethylene HDPE. In the process of the bag construction mechanical resistance of polyethylene HDPE was used, as well as its resistance to chemical factors and low temperatures. Thanks to the separation of containers for melting ice and for the kidney, possible becomes unrestrained performance of both venous and arterial anastomosis independently of existing operative conditions. Thanks the separation of container for kidney without liquid was eliminated of the diffusion of organic additives from polyethylene material into liquid and renal [11,12]. The renal bag Application of the described

topniejący lód i nerkę możliwe jest swobodne wykonywanie zespoleń zarówno żylnych jak i tętniczych niezależnie od zaistniałych warunków śródoperacyjnych. Zbiornik na nerkę pozbawiony jest płynu. Dzięki temu nie ma możliwości przenikania związków chemicznych ze ściany worka i ich ewentualnego działania na przeszczepianą nerkę [11, 12]. Zastosowanie opisanej modyfikacji całkowitej eliminacji ciepłego niedokrwienia umożliwia precyzyjne wykonanie naczyniowego etapu transplantacji nerki, co ma również korzystny wpływ na funkcjonowanie przeszczepionej nerki.

Wniosek

Dzięki zastosowanej metodzie eliminacji czasu ciepłego niedokrwienia z użyciem zbiornika polietylenowego HDPE można spodziewać się lepszej funkcji narządu po przeszczepie.

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modification of the total elimination of warm ischemia allows for the precise performance of the vessel stage of renal transplantation, which exerts also a beneficial effect on the graft function.

Conclusion

Due to the applied method of the elimination of the warm ischemia with use of the polyethylene bag HDPE one can expect better organ function after transplantation.

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CHARAKTERYSTYKA PASYWOWANYCH POWIERZCHNI STOPÓW NiTi WYKAZUJĄCYCH EFEKT PAMIĘCI KSZTAŁTU

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Wstęp

Stopy NiTi stały się jednym z ważniejszych materiałów pozwalających przezwyciężyć wiele trudności technologicz-

SURFACE CHARACTERIZATION OF NiTi SHAPE MEMORY ALLOY AFTER PASSIVATION

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[Engineering of Biomaterials, 37, (2004), 32-35]

Introduction

NiTi alloys have become an important material, which allows overcoming a wide range of technical and designing

nych i konstrukcyjnych związanych z miniaturyzacją urządzeń oferując tym samym bezinwazyjne i mniej bolesne prowadzenie operacji medycznych [1].

Pomimo szeregu wstępnych wyników badań klinicznych potwierdzających celowość stosowania stopów NiTi jako materiału na implanty, w tym również brak negatywnych skutków wywieranych na organizm ludzki, co pozwoliło na osiągnięcie konsensusu biofunkcjonalności i biokompatybilności, nadal istnieją obawy że powstające w wyniku korozji jony niklu mogą powodować niepożądane skutki uboczne [2].

Odporność korozyjna stopów metali jest oparta na zjawisku pasywacji wynikające z utleniania się metali na ich powierzchni. Powierzchnia stopu NiTi składa się w przeważającej mierze z tlenków tytanu - TiO_2 oraz w mniejszych ilościach z tlenków niklu (NiO i Ni_2O_3) i metalicznego Ni [3]. W zależności od metody pasywacji skład chemiczny powierzchni jak również i ilość niklu zmieniają się w szerokim zakresie [4].

Sposób przygotowania powierzchni stopu NiTi ma istotny wpływ na komórki limfocytowe śledziony szczurów. Obróbka powierzchni NiTi z zastosowaniem H_2O_2 powoduje jej toksyczność. Jednakże zastosowanie do pasywacji pary wodnej w autoklawie nie wykazuje toksycznego działania implantów. Wyjaśnieniem tego były różnice stężenia niklu w spasywowanej warstwie od 0.4 do 27% w zależności od przyjętej metody pasywacji. [4].

Dobra odporność korozyjna i biologiczna wynika z jednolitości cienkich, amorficznych warstw wytwarzanych na podłożach trawionych i poddanych działaniu parzy wodnej. ASTM zaleca pasywację elektrochemiczną w celu uzyskania warstw amorficznych, które są wolne od granic ziaren i dyslokacji, wykluczając przyczynę elektrochemicznego przebicia warstwy [5].

Celem prezentowanej pracy było otrzymanie amorficznych warstw TiO_2 w wyniku pasywacji stopu w strumieniu pary wodnej oraz zbadanie jej struktury, jednorodności i odporności na korozję.

Materiał i metodyka badań

Płytki stopu NiTi (50.6%at Ni) dostarczone przez firmę AMT (Belgia), po mechanicznym wypolerowaniu, były poddawane działaniu strumienia pary wodnej w autoklawie, w temperaturze 130°C przez 10, 20 i 30 minut.

Struktura pasywowanych warstw była badana metodą: rentgenowskiej reflektometrii (XRR), rentgenowskiej spektroskopii fotoelektronów (XPS) oraz techniką wysokorozdzielczej mikroskopii elektronowej (HREM).

Odporność na korozję wytworzonych warstw była sprawdzana w fizjologicznym roztworze Tyroda w cyklicznym potencjodynamicznym polaryzatorze.

Wyniki badań

W TABELI 1 zestawiono wyniki badań z rentgenowskich pomiarów reflektometrycznych - grubości warstwy tlenkowej, chropowatości powierzchni, chropowatość granicy rozdziału, gęstości warstwy tlenkowej i podłoża. W celu przeprowadzenia obserwacji amorficznej warstwy TiO_2 jej spójności z podłożem NiTi jak również i jednorodności przygotowano cienkie folie z przekrojów poprzecznych płytek. Zmierzona grubość amorficznych warstw tlenkowych pozostaje w dobrej zgodności z wynikami otrzymanymi metodą rentgenowskiej reflektometrii. Ciągłe przejście pomiędzy amorficzną warstwą tlenkową a krystalicznym podłożem przedstawiono na RYS. 1.

problems related to the miniaturisation of medical devices and offers less invasive, and therefore less traumatic medical procedures [1].

Despite the fact that most previous studies showed good clinical results of nickel-titanium implants and it looks that the Nitinol has no deleterious effects on human body, here is still some concern that freed due to corrosion nickel ions may cause undesirable side-effects [2].

The corrosion resistance of metal alloys is based on passivation phenomena witch is due to a metal oxide layer on the surface. The surface of NiTi consists mainly of stable titanium oxides TiO_2, smaller amount of nickel oxides (NiO and Ni_2O_3) and metallic Ni, while nickel-titanium constitutes the inner layer [3]. Depending on the passivation method, the surface chemistry and the amount of nickel may vary over a wide range [4].

The NiTi surface preparation has shown a critical effect on the cells of ratsplenocytes. The NiTi surface treatment with H_2O_2 caused a toxic effect comparable to that of pure nickel. However the treatment in water steam by autoclaving was clearly non-toxic. The explanation for this was that the nickel concentration at the surface could vary from 0,4 to 27 % depending on the passivation method [4].

A uniformly amorphous surface film creating at chemically etched and boiled in water substrates contributes to the excellent corrosion resistance and biological response. ASTM recommends electrochemical passivation to obtain an amorphous layer which is free of grain boundaries and dislocations and preclude electrochemical breakdown [5].

The aim of this work was to obtain an amorphous TiO_2 surface layer after passivation in water steam using the autoclaving process and to study the structure of this layer, its homogeneity and corrosion resistance.

Experimental

Flat specimens of the NiTi alloy (at 50,6 at. % Ni content the A_f =10°C) delivered by AMT (Belgium) after mechanical polishing were created by autoclaving in water steam at 130°C for 10, 20 and 30 minutes.

The structure of the passivated layers was studied using the X-ray reflectivity (XRR), X-ray photoelectron spectroscopy (XPS) methods and HREM imaging technique.

The corrosion resistance of the passivated surface was tested in the physiological Tyrod solution using the cyclic potentiodynamic polarization.

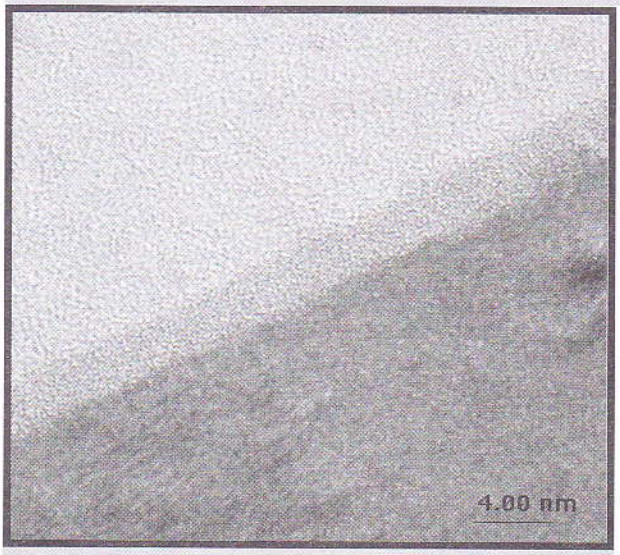
Results

The determined from X-ray reflectivity measurement oxide layer thickness, surface roughness, interface roughness and density of the oxide layers metal matrix are put together in TABLE 1. In order to observe the amorphous TiO_2 layers and their coherency with NiTi matrix as well as their homogeneity thin foils for HREM were prepared from the cross section of the flat specimens. The measured thickness of the amorphous oxide layer is in good agreement with the X-ray reflectivity results. A continuous transition from the amorphous layer to the crystalline alloy matrix is visible (FIG. 1).

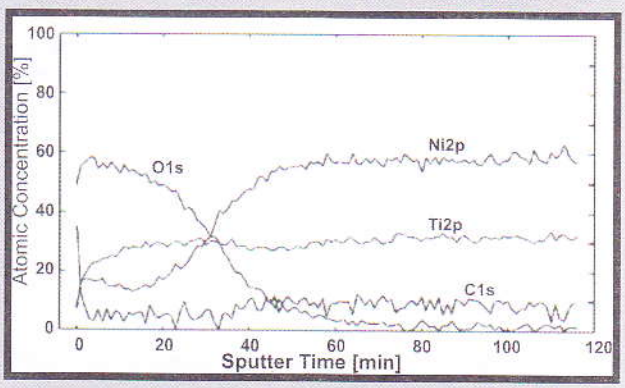
The structure of the oxide layer after passivation was determined using XPS method. The depth profile of the oxide layer for the specimen autoclaved at 130°C for 30 min. is shown in FIG. 2. The oxygen profile characterises the depth of the oxide layer with low level of the Ni concentration. In FIG. 3 the 2p Ti and Ni spectra of this specimen are shown for different detection angles corresponding to increasing depth information. The strong peak on the 2p Ti spectrum

Czas pasywacji Oxidation time [min]	Grubość warstwy tlenku Oxide thickness [nm]	Chropowatość powierzchni Surface roughness [nm]	Chropowatość granicy rozdziału Interface roughness [nm]	Gęstość tlenku Oxide density [g cm^{-3}]	Gęstość NiTi NiTi density [g cm^{-3}]
10	3.40	0.11	0.25	3.95	6.3
20	3.06	0.55	1.7	3.75	6.3
30	3.41	0.12	0.15	4.35	6.3

TABELA 1. Charakterystyka warstw otrzymana metodą rentgenowskiej reflektometrii.
TABLE 1. The results of the XRR measurements.

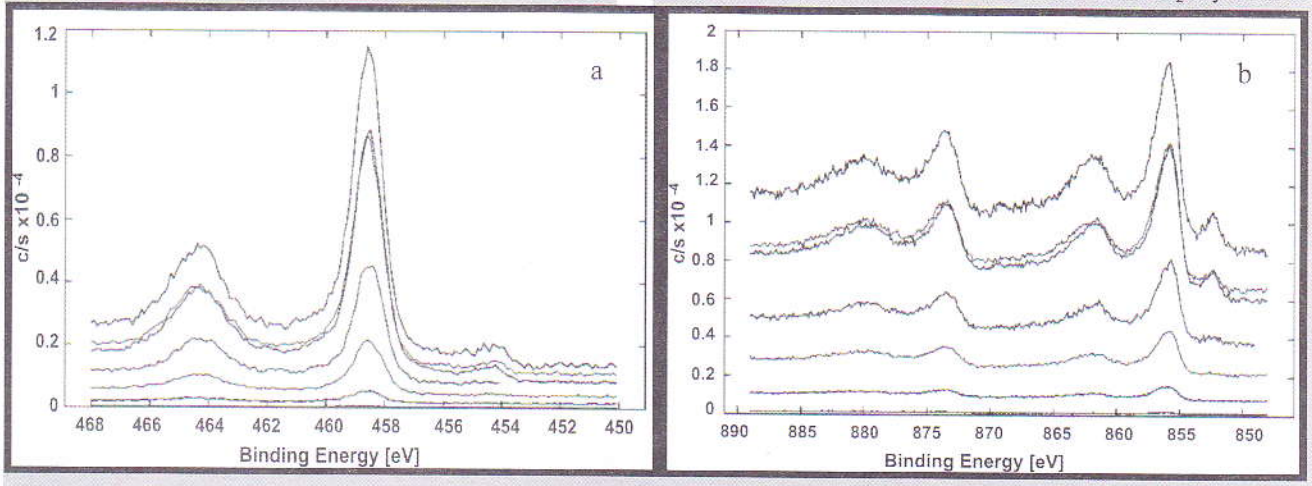


RYS. 1. Wysokorozdzielczy obraz mikroskopowy warstwy amorficznego tlenku.
FIG. 1. HREM image of the amorphous oxide.



RYS. 2. Profil głębokości warstwy tlenku dla próbki pasywowanej w 130°C w czasie 30 minut.
FIG. 2. Depth profile of the oxide layer for the specimen autoclaved at 130°C for 30 min.

(FIG. 3a) corresponds to the TiO_2 phase thus the amorphous layer can be identified as TiO_2. The intensity of the Ni 2p spectra (FIG. 3b) increases strongly with the detection angle. It may be concluded that below the TiO_2 layer there



RYS. 3. Widma 2p dla Ti (a) oraz Ni (b) rejestrowane pod różnymi kątami dla próbki pasywowanej w 130°C w czasie 30 minut.
FIG. 3. The 2p spectra of Ti (a) and Ni (b) for different detection angles of a specimen autoclaved at 130°C for 30 min.

Strukturę powierzchni warstwy tlenku uzyskanej w wyniku pasywacji badano również metodą XPS. Głębokość profilu warstwy tlenkowej wytworzonej w temperaturze 130°C w czasie 30 minut przedstawiono na RYS. 2. Profil wyznaczony dla tlenu charakteryzuje głębokość warstwy

exists a transition layer with nickel oxides. The samples oxidised at 130°C for 30 minutes have shown the best results of the corrosion resistance The established values are E_{corr} = +33 mV and breakdown potential E_{br} = +1227 mV. The presented here values are better than those presented

tlenkowej z niską zawartością Ni.

Na RYS.3 zostały przedstawione widma 2p dla Ti i Ni rejestrowane dla różnych kątów detekcji, co odpowiadało zwiększeniu głębokości penetracji. Dzięki obecności wysokiego maksimum widocznego na widmie 2p dla Ti (RYS. 3a), które jest charakterystyczne dla fazy TiO_2 można było zidentyfikować fazę amorficzną jako TiO_2. Natężenie widm 2p dla Ni (RYS. 3b) zwiększa się w miarę wzrostu kąta detekcji. Może to świadczyć o obecności tlenku niklu w przejściowej warstwie znajdującej się tuż poniżej warstwy TiO_2.

Otrzymane w autoklawie amorficzne warstwy tlenkowe na płytkach wykonanych ze stopu NiTi poddano badaniom odporności na korozję. Najlepsze wyniki w badaniach odporności na korozję uzyskano dla próbki stopu NiTi pasywowanego w temperaturze 130°C w czasie 30 minut. Wartość E_{corr} wynosiła +33 mV, natomiast potencjału przebicia (E_{br}) +1227 mV. Uzyskane wartości są znacznie korzystniejsze niż podawane w literaturze dla próbek pasywowanych elektrochemicznie.

Wnioski

- Podczas pasywowania w autoklawie można otrzymać cienką warstwę (~ 3 nm) TiO_2, której obecność zwiększa odporność na korozję
- Warstwa TiO_2 jest gładką, amorficzną warstwą nie wykazującą obecności atomów niklu
- Najwyższą wartość potencjału przebicia (E_{br} = +1227 mV) wykazują pasywowane warstwy wytworzone w autoklawie w temperaturze 130°C w czasie 30 minut

for electropolished passivation.

Conclusions

- The surface passivation by autoclaving ensures a thin (~ 3 nm) TiO_2 oxide film which is responsible for high corrosion resistance.
- The TiO_2 oxide layer is amorphous and shows low roughness and lack of nickel on the surface.
- After passivation at 130°C for 30 min. the highest breakdown potential (E_{br} = 1227 mV) is obtained.

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OCENA PEŁZANIA ELASTOMERÓW TERMOPLASTYCZNYCH W WARUNKACH DYNAMICZNYCH

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Wstęp

Materiał pracujący w warunkach cyklicznych obciążeń wymagana monitorowania procesów odkształceń i postępującego niszczenia materiału (zmęczenia). Wiele obciążeń w układach biologicznych ma charakter cykliczny (np. ścięgna), które wykazują zależne od czasu właściwości lepkosprężyste w odniesieniu do zmian sztywności, pętli histerezy czy pełzania [1, 2]. Gdy materiał polimerowy poddawany jest działaniu obciążeń, zachodzi w nim szereg procesów chemicznych i reologicznych. Efekt nałożenia się konkurujących procesów zależy od wielu czynników, takich jak temperatura, środowisko czy podstawowe właściwości molekularne polimeru [3]. W konwencjonalnych testach zmęczeniowych, próbka poddawana jest cyklicznym obciążeniom, podczas których wyznacza się liczbę cykli

VALUATION OF CREEPOF THERMOPLASTIC ELASTOMERS IN DYNAMIC CONDITIONS

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Introduction

If the materials are subjected to cyclic loads (fatigue) then characterisation of deformation and fracture properties of materials is of great interest. Many loads are cyclic in biological objects such in the case of tendons which experience loading patterns in vivo and show time-dependent viscoelastic properties in terms of change in stiffness, hysteresis loop or creep behaviour [1, 2]. When a polymeric material is subjected to an applied stress, a great variety of chemical and rheological events may occur in a given polymer. The net effect of the several competing processes depends on many factors, including temperature, environment, and basic molecular properties of the polymer [3]. In a conventional fatigue test, the specimen is subjected to cyclic loading and the number of cycles to failure is monitored [4]. The results are then presented as S-N curves, i.e. stress versus number of cycles to failure. Another testing approach is the hysteresis method which additionally gives

potrzebną do zniszczenia materiału [4]. Wyniki są przedstawiane w postaci charakterystycznych wykresów typu S-N, tzn. naprężenie w funkcji liczby cykli potrzebnych do zniszczenia materiału.Inną techniką badawczą jest metoda pętli histerezy, która dodatkowo dostarcza informacji o zmianach strukturalnych zachodzących w materiale. Różne właściwości są mierzone w tym samym czasie: naprężenie, amplituda odkształceń, wielkość absorbowanej i rozproszonej energii, tłumienie materiału oraz przebieg procesu pełzania w warunkach dynamicznych [5]. Metoda ta jest szczególnie pożyteczna w badaniach miękkich materiałów, takich jak elastomery termoplastyczne lub kauczuki. Stosując szybką metodę wyznaczania wartości naprężeń (w tzw. metodzie skokowo narastających obciążeń [6]) można ocenić proces "dynamicznego pełzania" w warunkach sinusoidalnie zmiennych obciążeń (obciążeń dynamicznych).W pracy przedstawiono ocenę właściwości zmęczeniowych nowego elastomeru termoplastycznego do zastosowań biomedycznych (czasowa proteza ścięgien zginaczy palców ręki), poli(alifatyczno/aromatycznego-estru) (PED) oraz stosowanego w medycynie poli(etero-uretanu) ze szczególnym uwzględnieniem procesu pełzania w warunkach dynamicznych.

Część doświadczalna

W pracy badano kopolimer PED zawierający 26% wag. poli(tereftalanu butylenu) (PBT) i 74% wag. dimeryzowanego kwasu tłuszczowego (FDA), otrzymany metodą transestryfikacji i polikondensacji w stopie, opisaną we wcześniejszych publikacjach [7, 8]. Poli(etero-uretan) (PU) o twardości Shora A 80 (Pellethane 2363-80A) otrzymano z firmy Dow, Niemcy. Próbki do badań statycznych i dynamicznych (wiosełka typu S2) przygotowano metodą wtrysku pod ciśnieniem ok. 50 MPa. Quasi-statyczne badania wytrzymałościowe wykonano w temperaturze pokojowej na maszynie wytrzymałościowej Instron TM-M, wyposażonej w głowicę 500 N przy szybkości przesuwu trawersu 100 mm/min. Do oceny właściwości zmęczeniowych kopolimeru PED i PU jako materiału odniesienia zastosowano maszynę serwohydrauliczną wyposażoną w cyfrowy sterownik (Instron 8400/8800) i pakiet do analizy pętli histerezy [6]. Maszyna serwohydrauliczna była wyposażona w głowicę 200 N oraz cylinder 10 kN. Stanowisko doświadczalne umożliwiało minimalizację przesunięcia sygnału naprężenia względem odkształcenia poniżej 20 μs. Podczas testu dynamicznego pełzania (SLT) próbki były poddawane sinusoidalnym odkształceniom o zadanej wartości naprężenia przy częstotliwości 1 Hz. Nie obserwowano histeretycznego nagrzewania próbki na jej powierzchni. Maksymalną wartość naprężenia wyznaczono na podstawie metody stopniowo narastających naprężeń (SILT) (TABELA 1). Naprężenie utrzymywano przez 100 000 cykli (tempe-

information about structural changes of the material. Different properties can be determined simultaneously - stress, strain amplitude, stiffnesses and stored and lost energies, material damping and the cyclic creep behaviour [5]. This method is particularly useful for evaluation of fatigue properties of soft materials such as thermoplastic elastomers or rubbers. With use of rapid method for determination of the amount of stress (so called stepwise increasing load test [6]), it is possible to evaluate the "dynamic creep" of a material when a sinusoidal force pattern (dynamic loading) is applied.In this study, evaluation of fatigue properties of novel thermoplastic elastomer for biomedical applications (temporary prosthesis for finger flexor tendon), poly(aliphatic/aromatic-ester) (PED) and a medical grade poly(ether-urethane) will be presented with special focus on "dynamic creep" behaviour.

Experimental

The synthesis method of PED copolymer containing 26wt% poly(butylene terephthalate) (PBT) and 74wt% dimerized fatty acid (DFA), involving transesterification and polycondensation from the melt has been described in previous publications [7, 8]. Poly(ether-urethane) (PU) of hardness Shore A of 80 (Pellethane 2363-80 AE) was received from Dow company (Germany). Samples for tensile and fatigue testing were prepared by injection moulding at a pressure of around 50 MPa. The quasi-static tensile data were collected at room temperature with an Instron TM-M tensile tester, equipped with a 500 N load cell, at a crosshead speed of 100 mm/min. A servo-hydraulic test machine with a digital controller (Instron 8400/8800) and a software package for the evaluation of the hysteresis loop [6] was used to study the PED copolymer and silicone elastomer as a reference material. A servo-hydraulic test machine was equipped with a 200-N load cell and 10 kN cylinder. A possible phase shift between stress and strain signal was minimized below 20 μs by the experimental set-up. In a single load test (SLT) the specimens were subjected to a stress controlled sinusoidal oscillation. The frequency, f, was in a range of 1 Hz and no hysteretic heating was detected at the surface of the specimen. The maximum stress was set at a value preselected from the stepwise increasing load testing (SILT) (TABLE 1). The stress was kept constant during a period of 100 000 cycles in air at 24°C. The digital controller was used to keep the load level constant with an accuracy of 5 %. The load ratio, R, was 0.1. This load ratio indicates that sinusoidal oscillations were cyclic repeated in tension mode at minimum load ten times lower than maximum load.

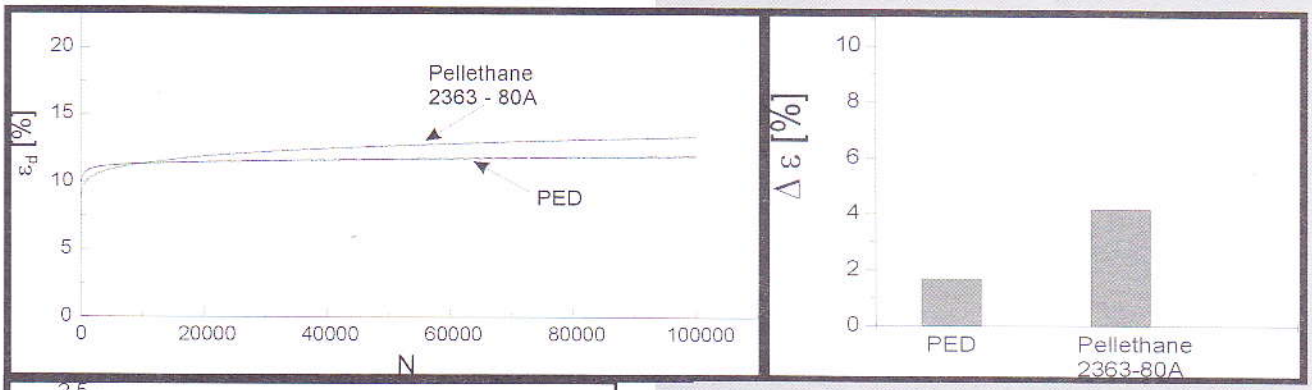
Results and discussion

The results from the quasi-static tensile measurements and

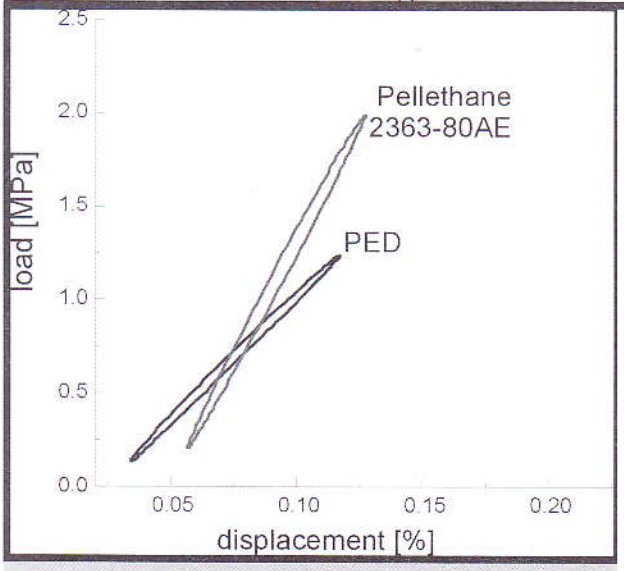
Sample code	PBT (wt.-%)	DFA (wt.-%)	E_{mod} [MPa]	σ_Y [MPa]	σ_r (UTS) [MPa]	ε_r [%]	T_m [°C]	$?_L$ [MPa]
PED (80*)	26	74	14 ± 0.7	-	5 ± 0.2	500 ± 60	152	1.25
Pellethane 2363 –80AE	80*		20 ± 6.1	-	21 ± 3.0	630 ± 50	195	2.0

* - hardness Shore A
E_{mod} – Young's modulus;
σ_r - stress at break;
σ_Y – yield stress;
ε_r - elongation at break;
σ_L – load values for dynamic creep test
T_m – melting point determined on a Böethius apparatus

TABELA 1. Statyczne właściwości wytrzymałościowe kopolimeru PED i materiału odniesienia. Szybkość przesuwu trawersu 100 mm/min.
TABLE 1. Static tensile properties of PED copolymer and a reference material. Cross-head speed of 100 mm/min.



RYS. 1. a) Krzywe pełzania dynamicznego (ε_d) kopolimeru PED i materiału odniesienia; wartości obciążeń są zaznaczone dla każdego polimeru; b) wartości bezwzględnego pełzania ($\Delta\ \varepsilon$) dla kopolimerów. Częstotliwość pomiarów: 1 Hz, liczba cykli: 100 000. T= 24°C.
FIG. 1. a) Dynamic creep (ε_d) curves of PED copolymer and a reference material; loading patterns are indicated for each polymer; b) absolute creep ($\Delta\ \varepsilon$) values for these copolymers. Test frequency: 1 Hz, number of cycles (N): 100 000. T = 24°C.



RYS. 2. Pętla histerezy kopolimeru PED i materiału odniesienia dla cyklu o numerze 50 000.
FIG. 2 Hysteresis loops of PED copolymer and a reference material taken at cycle number 50 000.

ratura 24°C). Stały poziom obciążenia z dokładności 5% był utrzymywany przez cyfrowy sterownik. Współczynnik amplitudy cyklu, R = 0.1. Oznacza to, że sinusoidalne oscylacje były cyklicznie powtarzane przy rozciąganiu próbki przy minimalnym obciążeniu dziesięć razy mniejszym niż obciążenie maksymalne.

Wyniki i dyskusja

Wyniki wytrzymałościowych badań quasi-statycznych oraz wartości obciążeń uzyskane z metody stopniowo narastających naprężeń przedstawia TABELA 1. RYSUNEK 1 przedstawia krzywe dynamicznego pełzania kopolimerów PED i Pellethane podczas testu dynamicznego pełzania (SLT) przy częstotliwości 1 Hz w temperaturze 24°C. "Pełzanie dynamiczne" zależy nie tylko od ruchliwości fragmentów łańcucha makrocząsteczki i krystaliczności, ale również od obecności wiązań drugorzędowych [9]. Kopolimer multiblokowy zawierający dimeryzowany kwas tłuszczowy w segmentach giętkich i wykazujący mikroseparację fazową nanostruktury charakteryzuje się małą wartość natychmiastowego odkształcenia elastycznego i małą wartość pełzania (ok. 1.8%) po 100 000 cykli. Kopolimer PED jest bardziej odporny na pełzanie w porównaniu do kopolimeru poliuretanowego (generalnie, poliuretany wykazują dobrą odporność na płynięcie pod obciążeniem dzięki obecności wiązań wodorowych stabilizujących strukturę). Słabszą odporność na pełzanie można interpretować zgodnie

load values for dynamic creep test are given in TABLE 1. FIG. 1 shows dynamic creep curves of PED and a Pallethane during constant loading (single load test, SLT) with a frequency of 1 Hz for a period of 100 000 cycles at 24°C. The "dynamic creep" depends not only on the mobility of the chain segments and crystallinity, but also on the strength of secondary bonds [9]. Multiblock copolymers containing dimerized fatty acid in the soft segments and showing microphase separated nanostructure shows very low value of immediate elastic deformation and very low creep (about 1.8 %) after 100 000 cycles. PED copolymer is more resistant to creep compared to polyurethane copolymer (polyurethanes usually show good resistance to creep due to the presence of strong hydrogen bonds stabilizing microstructure). Lower creep resistance of a polyurethane sample can be interpreted following suggestions [10, 11], that under the cyclic deformation the destruction of the hard segment domains and/or intermixing of the hard and soft segments can take place. FIG. 2 presents the comparison of hysteresis loop patterns of polymers tested under constant load. The much broader hysteresis loop of a polyurethane copolymer compared to PED copolymer can indicate structural changes, and in consequence induces high energy dissipation and very large creeping, as it was already discussed.

Conclusions

The hysteresis loop measurement method can successfully be applied to evaluate the fatigue properties of thermoplastic poly(aliphatic/aromatic-ester) (PED) multiblock elastomer in terms of creep behaviour. PED copolymer show much better creep resistance compared to poly(etherurethanes) when samples of similar hardness (Shore A 80) are compared. Evaluated properties can be very useful in the design of semicrystalline polymers which are subjected to a long duration loading.

z sugestią [10,11], iż podczas cyklicznych odkształceń następuje destrukcja twardych domen i/lub wymieszanie segmentów sztywnych i giętkich. RYSUNEK 2 przedstawia porównanie kształtu pętli histerezy nr. 50 000 dla polimerów testowanych przy stałym, cyklicznym obciążeniu. Znacznie szersze pole pętli histerezy kopolimeru poliuretanowego w porównaniu do kopolimeru PED może wskazywać na znaczne zmiany strukturalne, a w konsekwencji większe rozpraszanie energii i większe pełzanie, jak wspomniano wcześniej.

Wnioski

Metoda pętli histerezy może być z powodzeniem stosowana do oceny właściwości zmęczeniowych w odniesieniu do zjawiska pełzania dla poli(alifatyczno/aromatycznego-estru) (PED), będącego multiblokowym elastomerem termoplastycznym. Kopolimer PED wykazuje znacznie mniejsze pełzanie tj. mniejsze odkształcenie pod wpływem przyłożonego w warunkach dynamicznych obciążenia w porównaniu do kopolimeru poliuretanowego o porównywalnej twardości (Shore A 80).

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BADANIA DZIAŁANIA CYTOTOKSYCZNEGO POROWATYCH IMPLANTÓW KORUNDOWYCH Z DODATKIEM ANTYBIOTYKÓW

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Wstęp

Zakażenia kości trudno poddają się leczeniu i zazwyczaj wymagają długotrwałej kuracji antybiotykami. Miejscowe deponowanie odpowiednio skutecznego antybiotyku bezpośrednio do ogniska zakażenia zwiększa skuteczność leczenia i eliminuje skutki ogólnego, długotrwałego wpływu leku na cały organizm. Materiałem mogącym dobrze spełniać funkcję nośnika antybiotyku, szczególnie w chirurgii kości, są porowate implanty korundowe o wysokim stopniu biozgodności.. W Instytucie Szkła i Ceramiki w Warszawie opracowano porowate implanty korundowe w postaci pianki (45 ppi) z nośnikiem z hydroksypropylometylocelulozy HPMC firmy Fluka oraz porowate tworzywo korundowe z nośnikiem z poli(alkoholu winylu) PAW firmy Shin-Etsu. Z obu rodzajów materiałów przygotowano implanty z dodatkiem antybiotyków [1-4].

THE STUDY OF THE CYTOTOXYCITY EFFECTS OF THE POROUS CORUNDUM IMPLANTS CONTAINING ANTIBIOTICS

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Introduction

The bone infections pose the significant clinical problem and in most cases they need a long antibiotics treatment. The effectiveness of the treatment could be increased by the local deposition of the antibiotics, what additionally decreased the general effect of the long lasting antibiotic treatment in human patients. Among the materials which could be used as the effective drug carriers, especially in bone surgery, are porous corundum implants of the high biocompatibility. At the Institute of Glass and Ceramics in Warsaw two different materials were worked out: the porous corundum in the form of foam (45 ppi) with the hydroxypropylomethyl-cellulose carrier (HPMC made by Fluka Co.) and porous corundum with the poly(vinyl alcohol) carrier (PAW made by Shin-Etsu). From these both materials the implants containing antibiotics were prepared. [1-3].

The main target of the study was the comparative as-

Celem badań była ocena porównawcza w warunkach in vitro porowatych tworzyw korundowych z dodatkiem antybiotyków nowej generacji.

Badania cytotoksyczności są szybkim, wystandaryzowanym, czułym testem dla określenia czy dany materiał zawiera znaczące ilości, biologicznie szkodliwych substancji. Wysoka czułość tych testów jest wynikiem izolacji kultur komórkowych, a zatem braku mechanizmów ochronnych, które towarzyszą komórkom wewnątrz ciała. Pozytywne wyniki badań cytotoksycznych mogą być wczesnym ostrzeżeniem, że badany materiał zawiera więcej toksycznych substancji aniżeli dopuszcza poziom kliniczny [5].

Materiał i metoda badań

Badaniu działania cytotoksycznego poddano następujące implanty:

1. Pianki korundowe z nośnikiem HPMC bez antybiotyku lub z dodatkiem następujących ilości antybiotyków: 27,5 mg ceftriaksonu (Biotrakson, firmy Bioton) lub 21,5 mg cyprofloksacyny (firmy GZF Polfa Grodzisk Mazowiecki) lub 16 mg wankomycyny (Edicin, firmy Lek).
2. Porowate tworzywo korundowe z nośnikiem PAW bez antybiotyku lub z dodatkiem następujących ilości antybiotyków: 23,5 mg ceftriaksonu lub 26,5 mg cyprofloksacyny lub 16,5 mg wankomycyny.

Wszystkie implanty przygotowane były przez próżniowe nasączanie nośnika korundowego odpowiednim nośnikiem polimerowym zawierającym w/w antybiotyki. Bezpośrednio po nasączeniu próbki zamrożono (temp.-18°C), a następnie wysuszono metodą liofilizacji.

Badania działania cytotoksycznego przeprowadzono metodą bezpośredniego kontaktu na fibroblastach mysich 3T3 Balb/C założonych na płytkach Petriego w ilości 0,5 x 106 każda. Każdy materiał oceniano na 9 hodowlach komórek (po 3 na każdy dzień badania). Hodowle komórkowe inkubowano w temperaturze 37°C z dodatkiem 5% CO_2. Zmiany ilościowe i morfologiczne w hodowlach komórkowych oceniono po 24 , 48 i 72 h. Do oceny zastosowano barwienie czerwienią obojętną i błękitem trypanu. Wyniki ilościowe badań poddano analizie statystycznej.

Wyniki badań

Implanty korundowe bez antybiotyków

Po 24, 48 i 72 h w hodowlach po kontakcie z próbkami z pianki korundowej z nośnikiem HPMC i tworzywa korundowego z nośnikiem PAW komórki przylegały do podłoża i miały prawidłowe cechy morfologiczne. Nie stwierdzono aglutynacji, wakuolizacji, oddzielania od podłoża ani lizy błon komórkowych. Proliferacja komórek była prawidłowa i po 72 h komórki tworzyły kolonie. Ilość komórek żywych, po kontakcie z implantami w postaci pianki korundowej z HPMC, po 72 h była nieistotnie wyższa niż w hodowli macierzystej. Natomiast ilość komórek żywych, po kontakcie z porowatymi implantami korundowymi z PAW była nieistotnie niższa niż w hodowli macierzystej. Ilość komórek martwych po 72h, w przypadku pianki korundowej z HPMC wynosiła 2%, a w przypadku porowatego tworzywa korundowego z PAW - 3% całkowitej liczby fibroblastów, i była porównywalna z odsetkiem komórek martwych w hodowlach macierzystych -2% (RYS. 1, 2).

Implanty korundowe z dodatkiem ceftriaksonu

Po 24, 48 i 72 h w hodowli po kontakcie z próbkami z pianki korundowej z HPMC i tworzywa korundowego z nośnikiem PAW z dodatkiem ceftriaksonu komórki uległy silnemu obkurczeniu, aglutynacji i odklejeniu od podłoża. Pro-

sessment "in vitro" of the porous corundum implants with the new generation antibiotics added.

The evaluation of the cytotoxicity are quick, sensitive and standardized tests for the assessment of the biological impurities in the tested sample. The high sensitiveness of these tests is the result of isolation of cell culture, which as such have no defense mechanism which are present in the human body. The positive results of the cytotoxicity tests could be the early warning, that the material in question contains more toxic substances then this is allow at the clinical level [4].

Materials and methods

The study of cytotoxicity effects was carried on the following implant materials:

1. corundum foams with the HPMC carrier, without antibiotic or with the antibiotic of the following dosages: 27,5 mg cephtriaxon (Biotrakson, Bioton Co.) or 21,5 mg cyprophloxacine (GZF Polfa Co., Grodzisk Mazowiecki) or with 16 mg wankomycine (Edicin, Lek Co.);
2. porous corundum stuff with PAW carrier without antibiotic or with the antibiotics of the following dosages: 23,5 mg cephtriaxon (Biotrakson, Bioton Co.) or 26,5 mg cyprophloxacine (GZF Polfa Co., Grodzisk Mazowiecki) or with 16,5 mg wankomycine (Edicin, Lek Co.).

All implants were prepared by the vacuum soaking of the corundum materials with the proper polymer carrier with aforementioned antibiotics. Immediately after the materials have been soaked the samples were frozen at -18°C, and later all were dried by liophilisation.

Evaluation of the cytotoxicity was carried out by the method of direct contact on mouse fibroblast 3T3 Balb/C set up on Petri dishes at 0,5 x 106 each. Each sample was evaluated on 9 cell cultures (3 each tested day). The cell cultures were incubated at +37°C with 5% CO_2 added. The quantity and morphological changes in the cell cultures were assessed after 24, 48 and 72 hours. For this purpose the dyed methods with neutral red and trypane blue were used. The quantitative results of these tests underwent the statistical analysis.

Results

Corundum implants without antibiotics

After 24, 48 and 72 hours in the cell cultures which had the contact with the samples of corundum foam with HPMC carrier and with the porous corundum stuff with PAW carrier (both without antibiotics) all cells were properly adhered to the bed and had proper morphological character. No agglutination, vacuolization, separation from the bed neither lysis of the cell's walls were observed. Proliferation of the cell was correct and yet after 72 hours the cells formed proper colonies. The quantity cf the living cells after the contact with the implants in the form of corundum foam with HPMC carrier after 72 hours was higher then in the control cell culture but of no statistical importance. On the other hand the quantity of the living cells after contact with porous corundum implants was lower then in control culture, but as well of no statistical importance. Dead cell quantity after 72 hours of contact with corundum foam with HPMC was at the level of 2%, while in case of porous corundum with PAW at the level 0f 3% of the total number of fibroblasts and both was at the comparable level of dead cells counted for the control cultures -2% (FIG. 1, 2).

Corundum implants with cephtriaxon

After 24, 48 and 72 hours in the cell cultures which had

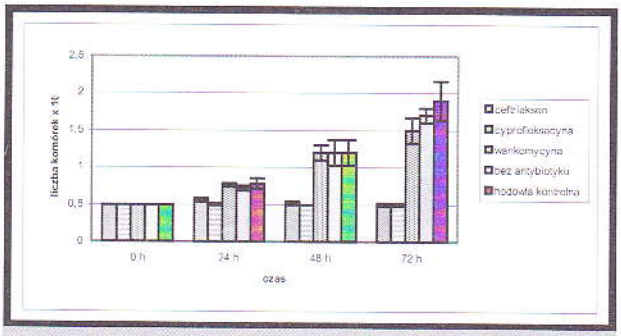
liferacja komórek w stosunku do wyjściowej liczby komórek była bardzo mała i wzrosła nieistotnie w porównaniu do wyjściowej liczby komórek. Liczba martwych komórek wyniosła 74% dla pianki korundowej z HPMC i 80% dla porowatego tworzywa korundowego z PAW. Po 48 h w hodowlach stwierdzono identyczne, jak po 24 h, zmiany morfologiczne komórek. Liczba martwych komórek wzrosła do 95 % w przypadku pianki korundowej z HPMC i do 100% w przypadku porowatego tworzywa korundowego z PAW. Po 72 h odsetek komórek martwych wyniósł 100, a cała hodowla uległa zniszczeniu .

Implanty korundowe z dodatkiem cyprofloksacyny

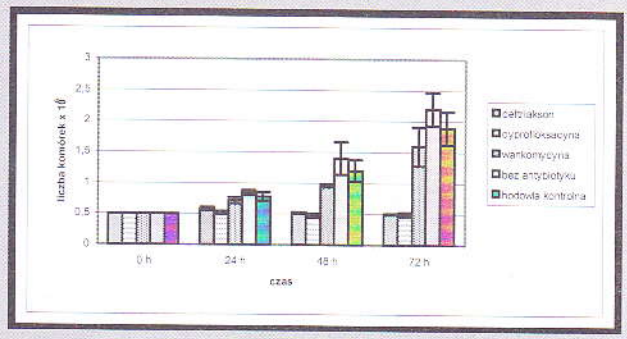
Po 24 h w hodowli po kontakcie z próbkami z pianki korundowej z nośnikiem HPMC i tworzywa korundowego z nośnikiem PAW z dodatkiem cyprofloksacyny komórki uległy silnemu obkurczeniu i aglutynacji. Tylko niewielki procent komórek był przyklejony do podłoża. Stwierdzono 80% komórek martwych w przypadku pianki korundowej z HPMC i 90% w przypadku tworzywa korundowego z PAW. Proliferacja komórek w stosunku do wyjściowej liczby komórek była bardzo mała i wzrosła nieistotnie w porównaniu do wyjściowej liczby komórek. Po 48h liczba martwych komórek wzrosła do 90 % w przypadku pianki korundowej z HPMC i do 100% w przypadku porowatego tworzywa korundowego z PAW. Po 72 h procent komórek martwych wyniósł 100, a hodowle uległy całkowitemu zniszczeniu. W każdym terminie badań, w polu widzenia, stwierdzono czarne masy, które świadczyły o wytrąceniu cyprofloksacyny.

Implanty korundowe z dodatkiem 16 mg wankomycyny

Po 24, 48, 72h w hodowlach po kontakcie z próbkami z pianki korundowej z nośnikiem HPMC i tworzywa korundowego z nośnikiem PAW z dodatkiem wankomycyny, komórki przylegały do podłoża i miały prawidłowe cechy morfologiczne. Nie stwierdzono aglutynacji, wakuolizacji, oddzielania od podłoża ani lizy błon komórkowych. We wszystkich hodowlach obserwowano dużą liczbę komórek w podziałach i ich prawidłową proliferację. Liczba komórek wzrosła istotnie w porównaniu do liczby wyjściowej oraz w porównaniu do prób z ceftriaksonem i cyprofloksacyną. Po 48 i 72 h w hodowlach stwierdzono nieistotnie wyższą liczbę komórek martwych w porównaniu do hodowli kontrolnej. We wszystkich hodowlach obserwowano pojedyncze powiększone komórki z ziarnistościami cytoplazmatycznymi. Po 24 h nie stwierdzono komórek martwych, a po 72 h



RYS. 2. Całkowita liczba fibroblastów mysich 3T3 Balb po kontakcie z implantami z porowatego tworzywa korundowego z nośnikiem PAW i z dodatkiem antybiotyków.
FIG. 2. The total number of mouse 3T3 Balb fibroblasts after the contact with porous corundum implants containing PAW and antibiotics.



RYS. 1. Całkowita liczba fibroblastów mysich 3T3 Balb po kontakcie z implantami w postaci pianki korundowej z nośnikiem HPMC i z dodatkiem antybiotyków.
FIG. 1. The total number of mouse 3T3 Balb fibroblasts after the contact with corundum foam implants containing HPMC and antibiotics.

the contact with the samples of corundum foam with HPMC carrier and with the porous corundum stuff with PAW carrier both with added cephtriaxon, cells were subject of the strong deformation and agglutination, and did not adhere to the bed. Cell's proliferation, as referred to the initial number, was very low and did not increase significantly. The quantity of dead cells was at the level of 74% for corundum foam with HPMC and even 80% for porous corundum with. After 48 hours the observed morphological changes of the cells were similar to those after 24h. The quantity of dead cells increased up to 95 % and 100% for corundum foam with HPMC and porous corundum with PAW respectively. After 72 hours the in both cases all cells were dead (100%) and the cultures were destroyed.

Corundum implants with cyprophloxacine

After 24 hours in the cell cultures with the contact with samples of corundum foam with HPMC and porous corundum with PAW, both with cyprophloxacine, the cells were subject of the strong shrinking and agglutination. Only small quantity of the cells were still adhered to the bed. The 80% of the dead cells were noted in the case of contact with the corundum foam with HPMC and up to 90% in case of contact with porous corundum with PAW. Proliferation of the cells in relation to their initial quantity was very low and slightly increased. After 48h the quantity of dead cells were at the level of 90% and 100% for corundum foam with HPMC and porous corundum with PAW respectively. After 72 h in both cases all cells were dead in 100% and the cultures were destroyed. In all investigation times, in the microscopic field of views the black, amorphic masses were observed, being the precipitated cyprophloxacine.

Corundum implants with 16 mg of wankomycine

After 24, 48 and 72 hours in the cell cultures which had the contact with the samples of corundum foam with HPMC carrier and with the porous corundum stuff with PAW carrier both with added wankomycine, cells were of the proper morphological characteristic and all adhered to the bed. No agglutination, vacuolization, separation from the bed neither lysis of the cell's walls were observed. In all cultures the high number of the mitotic cells were observed what was the proof of their proper and fast proliferation. The quantity of the cells in the culture in contact with tested materials increased significantly as compared to their initial number and as compared to the culture in contact with other tested antibiotics, i.e. cephtriaxon and cyprophloxacine. After 48 and 72 hours in the cultures the higher number of dead cells were noted as compared to control culture but of no

OVERVIEW OF BIOABSORBABLE BIOMATERIALS: DEVELOPMENT OF MULTIFUNCTIONAL OSTEOCONDUCTIVE DRUG-RELEASING HARD TISSUE FIXATION SCREWS

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Introduction

Further to achievement of reliable biocompatible hard reliable tissue bioabsorbable fixation devices, development of next generation was envisaged. There has been also an extensive research in development of slow-releasing drug systems. These two technologies thus were brought together, to develop devices, e.g. with dual function, hence the name "multifunctional" devices. For additional function, to address the problem of replacement of the bioabrorbale screw tracks with fibrous tissue, osteoconductivite agent was added.

Materials and methods

Bioaborbable polyesteric polymers (PLGA 80/20 or PLDLA 70/30) were used as the matrix material. Bioactive glass (BG) 93/13 was included to confer the osteoconductive function. In MFM-1, for infection-resistance function, ciprofloxacin (CF) was included in the implant. CF is bacteriocidal and it has a wide range of activity against osteomyelitis-causing bacteria, with good penetration to compact bone. In MFM-2, for the function of modification of tissue-reaction, agent-x1 was used. The composite was made into rods which were subsequently self-reinforced (SR), then machined into screws, and granules and sterilized using g-irradiation. Drug release, changes in molecular weight, in vitro degradation profiles, mechanical properties, and microstructure were evaluated. Effects of MFMs in vitro cell models were study the effect of the devices (on S. epidermides bacterial culture, attachment and biofilm formation; on chondrocytes, and on osteoblasts). In vivo models included the implantation in cranial bone of rabbits to assess tissue reactions, biodegradation and drug bone concentration. Biomechanical testing was also carried out using human cadaver bones (pull out tests). In an osteomyelitis model in rabbits, MFM granules were used.

Results

MFM-1

CF was released from the studied screws after 44 weeks (P/L/DL)LA) and 23 weeks (PLGA) in vitro. During this time drug release remained in range of 0.06 - 8.7 µg/ml/d (for P(L/DL)LA) and 0.6 - 11.6 µg/ml/d (for PLGA) after the start burst peak. The maximum release occurred in the 15th week (for P(L/DL)LA) and 8th week (for PLGA). CF remained bioactive throughout the in vitro drug release study. Initial mechanical properties of the screws are high and their application is easy. Measured initial shear strengths of the studied ciprofloxacin-releasing screws were 152 MPa (P/L/DL)LA) and 172 MPa (PLGA). Studied screws retain their mechanical properties at least 12 weeks (P(L/DL)LA) and 9 weeks (PLGA) in vitro at the level that ensures their fixation properties.

Histology

did not show much difference from the control plain PLGA screws except for some increased giant cells at some areas of the implantation site. Pull-out tests indicated that the early version of the MFM-1 type of screws have lower values as compared to controls. The inclusion of the bioactive glass leads to further drop in mechanical properties. Inhibition of bacterial growth, attachment and biofilm formation was significantly different than controls. In rabbit osteomyelitis model, healing was observed using MFM-1 antibiotic releasing devices.

MFM-2

Over 60 d, release The mode of the release curve followed close trend to that seen with MFM-1. A Peak was observed during the 1st 6h. SR has enhanced the release process as also didi g-sterilization further. SEM microstructure showed highly oriented SR structure and proper distribution of the drug agent. Study of mechanical properties is going on.

Comments

These are the first reliable MFM antibiotic-releasing screws in the world, that can add to surgeon's tools to combat against bone infection and its costly consequences. During degradation, MFM-1 screws progressively released the drug and retained sufficient mechanical properties over 2-3 months. For MFM-2, therapeutic levels were achieved and maintained for the time passed so far (61 d).

Conclusions

SR-P(L/DL)LA and SR-PLGA MF screws with appropriate drug release, structural, mechanical and biocompatibility properties can be produced. Clinical studies will be started in near future (MFM-1).

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Research funds from the Technology Development Center in Finland (TEKES, Biowaffle Project 40274/03 and MFM Project 424/31/04), the European Commission (EU Spare Parts Project QLK6-CT-2000-00487), the Academy of Finland (Project 73948) and the Ministry of Education (Graduate School of Biomaterials and Tissue Engineering) are greatly appreciated.

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