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Cell Cultivation on Porous Titanium Implants with Various Structures

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Abstract

The paper presents data on the cultivation of human dermal fibroblasts and rabbit mesenchymal stromal cells on two types of porous titanium implants, i.e., those with irregular pores formed by pressed titanium particles and those with regular pores formed by the cohesion of one-size titanium particles inside the implant. The goal of this study was to determine what type of titanium implant porosity ensured its strongest interaction with cells. Cells were cultivated on implants for 7 days. During this period, they formed a confluent monolayer on the implant surface. Cells grown on titanium implants were monitored by scanning electron microscopy. Fibroblasts interaction with implants depended on the implant porosity structure. On implants with irregular pores cells were more spread. On implants with regular pores fibroblasts enveloped particles and were only occasionally bound with neighboring particles by small outgrowths. There was no tight interaction of particles inside the implant. In implants formed by pressed particles, cells grow not only on surface, but also in the depth of the implant. Thus, we suppose that a tighter interaction of cells with the titanium implant and, supposedly, tissues with the implant in the organism will take place in the variant when the implant structure is formed by pressed titanium particles, i.e., cellular interaction was observed inside the implant. In implants with irregular pores, cells grew both on the surface and in the depth. Thus, cells exhibited more adequate interactions with irregular pore titanium implants in vitro and hopefully the same interaction will be true in tissues after the implantation of the prosthesis into the organism.

Keywords

titanium implant; fibroblasts; monolayer; topography; spreading; migration; adhesion

It is well known that titanium is applicable as a cell culture substrate. The biocompatibility of titanium implants was tested on various cells, e.g., fibroblasts, osteoblasts, chondrocytes, and stromal cells of bone marrow (Vehov et al., 2000; Deligianni et al., 2001; Ciolfi et al., 2003; Wang et al., 2004). To improve cell interaction with titanium implants their surface was modified with various treatments.

Some workers applied osteoinductive materials, such as apatites and calcium-phosphate compounds (Serro et al., 1997; Vehov et al., 2000; Deligianni et al., 2001; Pilliar et al., 2001; Wang et al., 2004; Koo et al., 2006). Others treated the surface of titanium implants with Hank's and Kokubo's saline solutions (Serro and Saramago, 2003) or a polymer coating (Reclaru et al., 2003; Park et al., 2007), or subjected the surface to chemical or thermal treatment (Fujibayashi et al., 2004).

To facilitate bone-tissue formation after engrafting the implant into rabbit bone, the titanium implant was treated with an EMD enamel matrix (Emdogain) (Stentport and Johansson, 2003); however, the results did not show a substantial improvement in bone formation around the titanium implant treated with EMD.

The preliminary treatment of titanium implants in vitro with silane, which promoted hydrophobic bonds with consecutive fibronectin adhesion, demonstrated that cells were more spread and had more focal contacts than cells seeded on untreated implants (Middleton et al., 2007).

There are a number of publications on treating titanium implants with various polymer materials. It was shown in experiments in vitro and confirmed in vivo that chitosan resorbable film combined with recombinant BMP-2 protein may be an attractive biomaterial for enhanced implant integration with cells in organism tissues (Lopez-Lacombe et al., 2006), whereas collagen type-I composition with BMP proteins and cultivated stromal cells of bone marrow may be utilized as spinal grafts (Minamide et al., 2005).

The preliminary treatment of titanium implants with fibroblasts cultured on polyglactin mesh facilitated bone tissue regeneration compared implants applied without mesh (Sparks et al., 2007). Endogenous implant coating with positively charged phosphorylcholine promoted the biocompatibility of medical devices with tissues (Susin et al., 2008). It was found that the expression of osteoblast phenotype in vitro was higher on a surface covered with RGD peptides than on an uncoated surface (Secchi et al., 2007).

Along with the application of various biological materials for improving the implant surface, studies were undertaken to evaluate physical factors that may facilitate the regeneration of bone tissue, e.g., various sterilization methods (Serro and Saramago, 2003), laser-treatment (Cho and Jung, 2003), electrical stimulation (Giannunzio et al., 2008), and titanium oxidation (Shibli et al., 2006).

Cell and implant interaction in vitro is monitored by cell adhesion, proliferation, and differentiation, as well as by the cell morphology and cytoskeleton (Ciolfi et al., 2003); additionally, other approaches are applied, including the adsorption of serum albumin on the implant surface (Serro et al., 1997; Deligianni et al., 2001; Wang et al., 2004); the value of the wetting angle (Serro and Saramago, 2003); the synthesis of extracellular proteins and proteoglycans by cells cultured on the implant surface (Ciolfi et al., 2003); and methods of scanning, confocal, and electron microscopy, as well as X-ray diffractometry (Park et al., 2007).

Implant biocompatibility in vivo was monitored by histological methods by soft and bone tissue formation on implant surface (Vehov et al., 2000; Cho and Jung, 2003; Fujibayashi et al., 2004; Koo et al., 2006; Shibli et al., 2007; Sparks et al., 2007). Contact between implants and tissues was evaluated by histochemical methods and with biomechanical criteria (Susin et al., 2008).

It is demonstrated that the extracellular matrix as a bioscaffold is implicated in recovery of structure and function of damaged tissue in organism. The major proteins of the extracellular matrix are collagen, fibronectin, laminin, nidogen, etc. The remodeling of extracellular matrix during wound healing stimulates angiogenesis, the renewal of circulating progenitor cells, the rapid degradation of scaffold, and cell adhesion and migration (Gorelik et al., 1994a, b, 1995a, b, 1998a, b, 2000; Paramonov et al., 1996; Blinova et al., 1997; Badyalak et al., 2000, 2001, 2002; Kalmykova et al., 2000, 2002; Cherepanova et al., 2001, 2003).

During the wound healing of a damaged skin surface, the extracellular matrix undergoes permanent modifications from a blood clot to the formation of granular and regenerating tissues. The functional cell activity during the reepithelialization in wound healing is regulated by various extracellular matrix elements and metalloproteinases (Voronkina et al., 1999; Gorelik et al., 2001). For example, collagen type I stimulates keratinocyte migration (Gorelik et al., 2001) and fibronectin, a major component of the extracellular matrix, is produced immediately after tissue damage and promotes keratinocyte migration in healing (Kim et al., 1992). Various laminins induce keratinocyte adhesion to substrate and semi-desmosome formation; i.e., they are engaged in the early stages of differentiation (O'Toole, 1997). Hence, the treatment of implanted construct with extracellular matrix components, presumably, may provide optimal conditions for implant interaction with organism tissues. The major matrix manufacturers are fibroblasts.

Leg amputation is accompanied with titanium rod implantation into amputation stump for subsequent prosthesis anchorage to the rod (Branemark, 1983; Eriksson, Branemark, 1994; Pitkin et al., 2006). Unsuccessful implantations frequently result from a lack of dense contacts of titanium implants with bone and skin cells around the rod (Pendegrass et al., 2006). To improve implant interaction with tissues in organism the implant surface required additional treatment. Experiments in vitro with cultured cells allow to screen implant surface modifications to create adequate contacts with cells.

The goal of the study was to elucidate how substrate topography (various porous structure of the titanium implant) in combination with implant treatment by extracellular matrix proteins, collagen I and fibronectin, more specifically, affects implant interaction with surrounding tissue and to decide the most optimal construct. Implant properties were tested with human dermal fibroblasts and rabbit bone marrow stromal cells cultured on the implant surface. The cell interaction with implants was monitored with scanning microscopy by cell morphology, the cell's capacity to achieve the confluent layer on implant surface and grow inside the implant.

MATERIALS AND METHODS

Implants

Four samples of porous titanium implants were provided by Poly-Orth International Company for testing. Two samples are flat discs (type I and II) with various porous structures and two samples are compact round rods (samples 2500 and 2600). Implants type I with irregular pores are produced from compact titanium particles (Fig. 1a). The porous structure of implants type II with regular pores is produced by adjacent beads of equal sizes (Fig. 1b). Type-II implants are characterized by uniform pores due to beads adjoined inside the implant. Before cell plating implants were sterilized by autoclaving for 40 min at 121°C and 15 psi pressure. Samples were tested in triplicate in each experiment. Cells were cultivated for 7 days on flat discs and up to 14 days on rods.

Preliminary treatment of implants: Flat discs

Before cell plating, sterile implants were incubated in cell growth medium (DMEM medium with 10% fetal calf serum) for 10 h. Control samples were untreated. 25×10^4 cells/ml dermal fibroblasts and 25×10^4 cells/ml rabbit bone-marrow stromal cells were seeded on treated implants placed in 3-cm Petri dishes.

Round rods (samples 2500 and 2600)

Samples were coated with type-I collagen or human plasma fibrin. Control samples were untreated. We chose these proteins for the coating because they are targets for the interaction

of a titanium prosthesis implanted in organism. For the coating, samples were immersed into type-I collagen (0.1 mg/ml) or human plasma fibrin (2 mg/ml) for 24 h at 4°C. Treated and untreated implants were embedded into a dermal equivalent, which is collagen gel with human dermal fibroblasts (1×10^5 cells/ml) (Fig. 2). Titanium samples in gel were cultivated according to the routine technique for 7 or 14 days with exchanging medium twice a week.

Cells

Human dermal fibroblasts were established from cells that migrated from fragments of fascial skin obtained from cosmetic surgery. Skin was cut into small, 0.1×0.1 cm, pieces. Fragments were placed in a Petri dish with DMEM medium supplemented with 10% fetal calf serum and incubated at 37°C in CO₂-incubator. In 3–4 weeks, the plate surface was covered with a confluent cell monolayer. The fibroblasts can be passaged several times; therefore, the number of cells may be increased. Bone-marrow stromal cells were obtained from rabbit pelvic bones with enzyme treatment. The cell's interaction with implants was evaluated by cell morphology, the pattern of their growth, and the depth of their penetration into the implant.

Discs

After 7 days, implants with cells were removed, cleansed of culture medium, fixed with 3.7% formaldehyde, and analyzed under a scanning electron microscope (GSM-35.7, Japan). Fibroblasts on type-I implants were stained with rhodamine-phalloidin to visualize the cytoskeleton and assessed by fluorescence microscopy to verify that the implant is covered with cells.

Rods

Dermal equivalents with rod samples were cultivated for 14 days. Samples fixed for 7 or 14 days were also analyzed by scanning electron microscopy.

RESULTS AND DISCUSSION

Cells actively proliferated on all implants tested.

Type-I implants (with pores irregular in size and arrangement)

After 7 days, cultured dermal fibroblasts produced a confluent monolayer of cells was well spread on the implant surface. Cells in areas adjacent to pores were connected by cellular bundles (Fig. 3). Scanning microscopy demonstrated that fibroblasts were grown both on the surface (Fig. 3a) and in the depth of the disc. Cells were found at a depth of 0.3–0.5, 0.8–1.0, and 1.3–1.5 mm in longitudinal implant fractures (Fig. 3b–3d).

Cells grown on these implants in a single experiment were stained with rhodamine-phalloidin to visualize the actin cytoskeleton by fluorescent microscopy and, therefore, to identify cells because transmission microscopy is inadequate for these dense substrates. It was found that the cytoskeleton in cells grown on three-dimensional substrates (implants) is distinguished from the cytoskeleton in cells grown on a two-dimensional plane, which is in accordance with the literature data (Ciolfi et al., 2003).

Rabbit bone-marrow stromal cells cultured on type-I implants are also well spread on the substrate and actively interacted with it (Fig. 4).

Type-II implants (with equal size and uniformly arranged pores)

Dermal fibroblasts seeded on these implants and cultivated under the same conditions (growth medium, inoculate dose, cultivation period) as plated on a type-I implant generated a confluent monolayer on the surface of beads that form the implant porous structure (Fig. 5). Only rare cellular contacts (bundles) between adjacent beads in the same or neighboring rows were observed.

The results show that human skin fibroblasts and rabbit bone marrow stromal cells tightly interact with the implants: cells adhere, migrate, proliferate and produce confluent layers on tested samples in all experiments. Cells grow both on the surface of the implant surface and in its depth. The cell confluent layer was different on implants with various porous structures (type I, Fig. 3; type II, Fig. 5), which is probably due to various implant surface topography. Well-spread fibroblasts on type-I implants (irregular pores) form a large bundle that overlaps the pore and attaches to the implant on its other side.

On the surface of type-II implants (pores equal in size and uniformly arranged), dermal fibroblasts form a confluent multilayer during the same culture period. Cells envelope the bead surface, but are not visible on adjacent beads and do not overlap with pores. Small bundles of cells are only occasionally observed between adjacent beads (Fig. 5). The preliminary treatment of the implant with growth medium does not substantially affect the cell growth and interaction with implants.

Rod samples 2500 and 2600 without cell seeding were tested by incubation in a dermal equivalent for 14 days. Samples were fixed in 7 or 14 days in culture.

No cells on the titanium surface or implant inside were revealed in 7 days. In 14 days cells were identified on the surface of sample 2500. Most cells were revealed on the surface of untreated and fibrin-treated samples. A few cells that probably migrated from the sample surface were observed on the inner edge of the sample fracture. In fibrin-treated and untreated samples, a small number of cells was noticed in the central part of the rod fracture with a greater number of cells in fibrin-treated samples.

Cells were visible on the surface of 2600 sample both treated and not treated with proteins and cultured with dermal equivalent (Fig. 6). The greatest number of cells was registered on a surface of untreated or fibrin-treated samples. Along the inner edge of sample fractures, cells presumably migrated from the sample surface were visible in similar quantities for both untreated and fibrin-treated samples (Fig. 7). The number of cells was considerably smaller in samples treated with collagen.

A small number of cells was observed in the central part of the rod in all samples. Unlike discs with plated cells, 14 days in culture is most likely insufficient for cells to migrate from dermal equivalent inside the rod. The appearance of cells on rod implants that were not seeded with cells before their incubation with dermal equivalent indicates their possible migration from the equivalent. Cells observed on the inner edge of the sample fracture in 14 days in culture (mostly in fibrin-treated samples) also indicate the migration of the cell from the equivalent on the implant surface and even inside the implant.

The greatest number of cells was registered on a rod surface treated with human plasma fibrin. Fibrin is supposedly a preferable substrate to collagen because the function of fibroblasts in healing damaged tissue in an organism is to remodel blood fibrin and synthesize collagen. This process is probably observed in our model.

Human dermal fibroblasts and rabbit bone marrow stromal cells capacity to adhere, spread, and proliferate on the tested titanium implants is in good agreement with the data of other

workers who applied titanium as a substrate for cell growth (Vehov et al., 2000; Deligianni et al., 2001; Cioffi et al., 2003; Wang et al., 2004). The tendency to improve the biocompatibility of titanium implants with surrounding organism tissues is evident. Various materials were examined for treating implants, including resorbed polymers (e.g., chitozan) in combination with proteins (BMP), which facilitated the cells' interactions with the substrate (Minamide et al., 2005; Lopez-Lacombe et al., 2006; Middleton et al., 2007); polyglactin mesh with growing fibroblasts (Sparks et al., 2007); positively-charged phosphorylcholine, which that is beneficial for cells and, therefore, improves the biocompatibility of medical devices with tissue in the organism (Susin et al., 2008); and RGD peptides (Secchi et al., 2007). We applied extracellular proteins (collagen and fibrin) for the preliminary treatment of titanium implants, which was more adequate for the organism and, consequently, more effective for the migration of cells from tissue than chemical, physical, or polymer treatments (Cho, Jung, 2003; Reclaru et al., 2003; Serro and Saramago, 2003; Stentport and Johansson, 2003; Fujibayashi et al., 2004; Park et al., 2007; Shibli et al., 2007; Giannunzio et al., 2008).

Experiments on cells cultured on titanium implants with various porosity structures clearly show that implant topography affect its interaction with cells. It is in accordance with the data on implant microtopography and the geometry effect (Proff et al., 2006; Ricci et al., 2007; Moreo et al., 2009).

The experimental model we used *in vitro* to study the interaction of the dermal equivalent with the titanium implant enabled us to predict the cell's interaction with the implant in the organism. Cells cultured on a porous titanium implant grow both on its surface and inside of it. It is important that the pattern of cell interaction with implants be defined by the porosity structure of the implant. Interactions between the cell and titanium implant is more evident in samples with nonuniform porosity (pressed material). Moreover, it was demonstrated that fibroblasts from dermal equivalents are able to migrate on the surface of immersed titanium samples and inside implants through the pores. Hence, it is possible to assume that the same implant interaction with surrounding tissues will take place in an organism when the implant is transplanted in the organism, which may facilitate the closer integration of the implant with adjacent tissues.

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References

- Blinova MI, Paramonov BA, Kukhareva LV, Gorelik BV, Nikitina YuM, Voronkina IV. Effect of Fibroblasts, Collagen, and Laminin on Wound Healing after Cutting off a Piece of Skin in Rats. *Byul Eksp Biol Med*. 1997; 124:229–232.
- Gorelik, YuV; Blinova, MI.; Pinaev, GP. The Effect of the Components of the Extracellular Matrix on the Spreading of Rat Keratinocytes in the Substrate During Cultivation in a Low-Calcium Medium. *Tsitologiya*. 1994; 36(12):1209–1212. [PubMed: 7544509]
- Gorelik, YuV; D'yakonov, IA.; Kukhareva, LV.; Blinova, MI.; Pinaev, GP. Effects of Extracellular Matrix Elements on Pseudopodial Activity of Rat Keratinocytes. *Tsitologiya*. 1998; 40(12):1037–1044. [PubMed: 10188218]
- Kalmykova NV, Cherepanova OA, Gorelik YuV, Voronkina IV, Blinova MI, Pinaev GP. Various Effects of Laminin-1 and Laminin-2/4 on Adhesion and Migration of Cultured Human Keratinocytes. *Tsitologiya*. 2002; 44(8):792–798. [PubMed: 12506671]
- Paramonov, BA.; Blinova, MI.; Nikitina, YuM; Gorelik, YuV; Voronkina, IV.; Kukhareva, LV.; Malakhov, SA.; Pinaev, GP. Effect of Fibroblasts Cultured *in vitro* and Extracellular Matrix Components on Cutaneous Wound Healing. *Materialy Mezhdunarodnogo simpoziuma "Novye*

- metody lecheniya ozhogov s ispol'zovaniem kul'tiviruemykh kletok kozhi"; Proc. Int. Symp. "New Methods in Treatment of Burns using Cultured Dermal Cells; Tula. 1996. p. 8
- Cherepanova OA, Kalmykova NV, Blinova MI, Pinaev GP. Beta-1 and Beta-4 Integrins, and Laminin 67 kDa Receptors in Interaction between A431 Cells and Laminin Isoforms. *Tsitologiya*. 2003; 45(3):271–276. [PubMed: 14520883]
- Yudintseva NM, Gorelik YuV, D'yakov IA, Kalmykova NV, Kukhareva LV, Blinova MI, Pinaev GP. The Use of Dermal Skin Equivalent for Wound Healing. *Tsitologiya*. 1999; 41(3–4):329.
- Blinova MI, Yudintseva NM, Kalmykova NV, Kuzminykh EV, Yurlova NA, Ovchinnikova OA, Potokin IL. Effect of Melanins from Black Yeast Fungi on Proliferation and Differentiation of Cultivated Human Keratinocytes and Fibroblasts. *Cell Biol Int*. 2003; 27:135–147. [PubMed: 12662970]
- Badylak SF, Meurling S, Chen M, Spivack A, Simmons O, Byre A. Resorbable Bioscaffold for Esophageal Repair in a Dog Model. *J Pediatr Surg*. 2000; 35:1097–1103. [PubMed: 10917304]
- Badylak SF, Park K, McCabe G, Yoder M. Marrow-Derived Cells Populate Scaffolds Composed of Xenogenic Extracellular Matrix. *Exp Hematol*. 2001; 29:1310–1318. [PubMed: 11698127]
- Badylak SF. The Extracellular Matrix as a Scaffold for Tissue Reconstruction. *Cell Dev Biol*. 2002; 13:377–383.
- Brånemark PI. Osseointegration and Its Experimental Studies. *J Prosthetic Dentistry*. 1983; 50:399–410.
- Cheng YL, Stanford CM, Wefel JS, Keller JC. Osteoblastic Cell Attachment to Hydroxyapatite-Coated Implant Surfaces *in vitro*. *Int J Oral Maxillofac Implants*. 1999; 14:239–247.
- Cherepanova, OA.; Kalmykova, NV.; Kuzminykh, EV.; Blinova, MI.; Pinaev, GP. The Contribution of YIGSR and IKVAV Peptides and $\beta 1$ Integrin Chain into the Process of Cell Interaction with Laminins. Abstracts of the 43th International Meeting of the European Tissue Culture Society "Cell Interaction and Cellular Complexity,"; Granada, Spain. 2001. p. 2-02
- Cho SA, Jung SK. A Removal Torque of the Laser-Treated Titanium Implants in Rabbit Tibia. *Biomaterials*. 2003; 24:4859–4863. [PubMed: 14530083]
- Ciolfi VJD, Pillar R, McCulloch C, Wang SX, Grynbas MD, Kandel RA. Chondrocyte Interaction with Porous Titanium Alloy and Calcium Polyphosphate Substrates. *Biomaterials*. 2003; 24:4761–4770. [PubMed: 14530073]
- Deligianni DD, Katsala ND, Koutsoukos PG, Missirlis YF. Effect of Surface Roughness of Hydroxyapatite on Human Bone Marrow Cell Adhesion, Proliferation, Differentiation and Detachment Strength. *Biomaterials*. 2001; 22:87–96. [PubMed: 11085388]
- Eriksson E, Brånemark PI. Osseointegration from the Perspective of the Plastic Surgeon. *Plast Reconstr Surg*. 1994; 93:626–637. [PubMed: 8115525]
- Fujibayashi S, Neo M, Kim HM, Kokubo T, Nakamura T. Osteoinduction of Porous Bioactive Titanium Metal. *Biomaterials*. 2004; 25:443–450. [PubMed: 14585692]
- Giannunzio GA, Speerli RC, Guglielmotti MB. Electrical Field Effect on Peri-Implant Osteogenesis: A Histologic and Histomorphometric Study. *Implant Dent*. 2008; 17:118–126. [PubMed: 18332765]
- Gorelik, YuV; Blinova, MI.; Pinaev, GP. Culture Substrate Influence Rat Keratinocyte Differentiation and Cytoskeleton Actin Distribution. *Cell Biol Int*. 1994; 18:435–440.
- Gorelik, JuV; Blinova, MI.; Diakonov, IA.; Kukhareva, LV.; Pinaev, GP. Role of Feeder Cells in Spreading and Cytoskeleton Organization of Newborn Rat Keratinocytes. *Cell Biol Int*. 1995; 19:59–64. [PubMed: 7613512]
- Gorelik, JuV; Paramonov, BA.; Blinova, MI.; Diakonov, IA.; Kukhareva, LV.; Pinaev, GP. Matrigel Increases the Rate of Split Wound Healing and Promotes Keratinocyte "Take" in Deep Wounds in Rats. *Cytotechnology*. 2000; 32:79–86. [PubMed: 19002969]
- Gorelik, JuV; Cherepanova, OA.; Voronkina, IV.; Diakonov, IA.; Blinova, MI.; Pinaev, GP. Laminin-2/4 from Human Placenta Is a Better Adhesion Agent for Primary Keratinocytes than Laminin-1 from EHS Sarcoma. *Cell Biol Int*. 2001; 25:395–402. [PubMed: 11401326]
- Hansson HA, Albrektsson T, Brånemark PI. Structural Aspects of the Interface between Tissue and Titanium Implants. *J Prosthet Dent*. 1983; 50:108–113. [PubMed: 6576146]

- Kalmykova, NV.; Cherepanova, OA.; Gorelik, JuV; Blinova, MI.; Pinaev, GP. Laminin 2/4 in Adhesion and Migration of Human Keratinocytes. Abstracts of XVIIth Meeting of the Federation of the European Connective Tissue Societies; 2000. p. J7
- Kim JP, Zhang K, Chen JD. Mechanisms of Human Keratinocyte Migration on Fibronectin: Unique Role of RGD Sites and Integrins. *J Cell Physiol.* 1992; 151:443–450. [PubMed: 1295896]
- Koo S, Konig B Jr, Allegrini S Jr, Yoshimoto M, Carbonari MJ, Mitri-Luiz FF. Titanium Implant Osseointegration with Calcium Pyrophosphate in Rabbits. *J Biomed Mater Res B Appl Biomater.* 2006; 76:373–380. [PubMed: 16184530]
- Lopez-Lacomba JL, Garcia-Cantalejo JM, Sanz Casado JV, Abarrategi A, Correias Magana V, Ramos V. Use of rhBMP-2 Activated Chitosan Films to Improve Osseointegration. *Biomacromolecules.* 2006; 7:792–798. [PubMed: 16529416]
- Middleton CA, Pendegrass CJ, Gordon D, Jacob J, Blunn GW. Fibronectin Silanized Titanium Alloy a Bioinductive and Durable Coating to Enhance Fibroblast Attachment *in vitro*. *J Biomed Mater Res Part A.* 2007; 83:1032–1038.
- Minamide A, Yoshida M, Kawakami M, Yamasaki S, Kojima H, Hashizume H, Boden SD. The Use of Cultured Bone Marrow Cells in Type I Collagen Gel and Porous Hydroxyapatite for Posterolateral Lumbar Spine Fusion. *Spine.* 2005; 30:1134–1138. [PubMed: 15897826]
- Moreo P, Garcia-Aznar JM, Doblare M. Bone Ingrowth on the Surface of Endosseous Implants Part 1: Mathematical Model. *J Theor Biol.* 2009; 260:1–12. [PubMed: 18762197]
- O'Toole EA, Marinkovich MP, Hoefler WK, Furthmayer H, Woodley DT. Laminin-5 Inhibits Human Keratinocytes Migration. *Exp Cell Res.* 1997; 233:330–339. [PubMed: 9194495]
- Park JW, Park KB, Suh JY. Effects of Calcium Ion Incorporation on Bone Healing of Ti6Al4V Alloy Implants in Rabbit Tibiae. *Biomaterials.* 2007; 28:3306–3313. [PubMed: 17462729]
- Pendegrass CJ, Goodship AE, Blunn GW. Development of a Soft Tissue Seal around Bone-Anchored Transcutaneous Amputation Prostheses. *Biomaterials.* 2006; 27:4183–4190. [PubMed: 16618500]
- Pilliar RM, Filiaggi MJ, Wells JD, Grynblas MD, Kandel RA. Porous Calcium Polyphosphate Scaffolds Substitute Applications—in vitro Characterization. *Biomaterials.* 2001; 22:963–974. [PubMed: 11311015]
- Pitkin M, Raykhtsaum G, Galibin OV, Protasov MV, Chihovskaya JV, Belyaeva IG. Skin and Bone Integrated Prosthetic Pylon: A Pilot Animal Study. *J Rehabil Res Dev.* 2006; 43:573–580. [PubMed: 17123195]
- Proff P, Kauschke E, Rumpel E, Bayerlein T, Dietze S, Fanghanel J, Gedrange T. The Survival and Proliferation of Fibroblasts on Orthodontic Miniscrews with Different Surface Treatment: An *in vitro* Study. *Folia Morphol (Warsz).* 2006; 65:78–80. [PubMed: 16783745]
- Reclaru L, Lerf R, Eschler PY, Blatter A, Mey JM. Evaluation of Corrosion on Plasma Sprayed and Anodized Implants, both with and without Bone Cement. *Biomaterials.* 2003; 24:3027–3038. [PubMed: 12895575]
- Ricci JL, Grew JC, Alexander H. Connective-Tissue Responses to Defined Biomaterial Surfaces, I. Growth of Rat Fibroblast and Bone Marrow Cell Colonies on Microgrooved Substrates. *J Biomed Mater Res, A.* 2007; 85:313–325. [PubMed: 17688290]
- Secchi AG, Grigoriou V, Shapiro IM, Cavalcanti-Adam EA, Composto RJ, Ducheyne P, Adams CS. RGDS Peptides Immobilized on Titanium Alloy Stimulate Bone Cell Attachment, Differentiation and Confer Resistance to Apoptosis. *J Biomed Mater Res A.* 2007; 83:577–84. [PubMed: 17503524]
- Serro AP, Fernandes AG, Saramago B, Lima J, Barbosa MA. Apatite Deposition on Titanium Surfaces. The role of Albumin Adsorption. *Biomaterials.* 1997; 18:963–968. [PubMed: 9212191]
- Serro AP, Saramago B. Influence of Sterilization on the Mineralization of Titanium Implants Induced by Incubation in Various Biological Model Fluids. *Biomaterials.* 2003; 24:47–49. [PubMed: 12417177]
- Shibli JA, Grassi S, Cristina de Figueiredo L, Feres M, Marcantonio E Jr, Iezzi G, Piattelli A. Influence of Implant Surface Topography on Early Osseointegration: A Histological Study in Human Jaws. *J Biomed Mater Res B, Appl Biomater.* 2007; 80:377–385. [PubMed: 16850464]

- Sparks MS, Kerns DG, Wilson TG, Hallmon WW, Spears R, Haghighat N. Bone Regeneration around Implants in the Canine Mandible with Cultured Fibroblasts in Polyglactin Mesh. *J Periodontol*. 2007; 78:1276–1287. [PubMed: 17608583]
- Stentport VF, Johansson CB. Enamel Matrix Derivative and Titanium Implants. An Experimental Pilot Study in the Rabbit. *J Clin Periodontol*. 2003; 30:359–364.
- Susin C, Qahash M, Hall J, Sennerby L, Wikesjö UM. Histological and Biomechanical Evaluation of Phosphorylcholine-Coated Titanium Implants. *J Clin Periodontol*. 2008; 35:270–275. [PubMed: 18269667]
- Vehov JWM, Spauwen PHM, Jansen JA. Bone Formation in Calcium-Phosphate-Coated Titanium Mesh. *Biomaterials*. 2000; 21:2003–2009. [PubMed: 10941922]
- Voronkina, IV.; Gorelik, YuV; Are, AF.; Kalmykova, NV.; Potokin, IL.; Pinaev, GP. The Peculiarities of Attachment and Spreading of Normal and Transformed Epithelial Human Cells on Different Extracellular Matrix Proteins. In: Skateris, GG.; Nicolson, GL., editors. *Intermolecular Cross-Talk in Tumor Metastasis*. Vol. 311. IOS Press; Amsterdam: 1999. p. 171-181.
- Wang J, Layrolle P, Stigter M, de Groot K. Biomimetic and Electrolytic Calcium Phosphate Coatings on Titanium Alloy: Physicochemical Characteristics and Cell Attachment. *Biomaterials*. 2004; 25:583–592. [PubMed: 14607496]

Fig. 1. Implant structure. (a) Type I implant (irregular pores); (b) type II implant (irregular pores); A, pore. Scale bar is 1000 μm . Here and in other figures, scanning-microscopy data are presented.

Fig. 2.
Dermal equivalent with fibroblasts in collagen gel (human skin derma analog in vitro).
F, fibroblasts (*arrows*). Obj. 10×.

Fig. 3.
Dermal fibroblasts cultured on type-I implant for 7 days.
On the implant surface (a); (b, c, d) at depth of 0.3–0.5, 0.8–1.0 and 1.3–1.5 mm,
respectively. A, pore; C, cells on the implant; arrow shows a cell between pores. Scale bar is
100 μ m.

Fig. 4. Rabbit bone marrow stromal cells on the surface of type I implant in 7 days in culture. Designations are the same as in Fig. 3. Scale bar is 10 μ m.

Fig. 5.
Dermal fibroblasts on the surface of type II implant in 7 days in culture.
Designations are the same as in Fig. 3. Scale bar is 1000 (a) or 10 (b) μm .

Fig. 6. Dermal fibroblasts on the surface of titanium rod (sample 2600) in 14 days of its cultivation in dermal equivalent. (a) No treatment (control); (b) collagen treatment; (c) fibrin treatment. Designations are the same as in Fig. 3. Scale bar is 10 μ m.

Fig. 7. Dermal fibroblasts in the depth of titanium rod (rod fracture). (a, b, c) Rod center; (d, e, f) rod edge; (a, d) control; (b, e) rod treated with collagen; (c, f) rod treated with fibrin. Designations are the same as in Fig. 3. Scale bar is 10 μ m.