Synopsis: Biomaterials are very often used as scaffold for regenerating tissue, either in vitro and to be implanted later, or directly into the defect in vivo. A successful biomaterial will integrate in the body without causing massive inflammation and/or fibrosis. Inflammation is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. When this healing process is out of balance, fibrosis can occur. Fibrosis can be designated as an abnormal healing process characterised by an excessive accumulation of extracellular matrix proteins (in particular collagen). This process alters the extracellular matrix structure and will eventually result in loss of function of the particular tissue.

In this symposium we want to discuss the effects biomaterials have on the behaviour of the cells seeded on or surrounding the biomaterial focussing on reactions related to inflammation, wound healing and fibrotic reactions.

(6.KP) THE FOREIGN BODY REACTION AGAINST GELATIN AND (NON-)CROSSLINKED COLLAGEN DISPLAY MAJOR DIFFERENCES

Bank RA (1), Harmsen MC (1), van Putten SM (1)

1. Department of Pathology & Medical Biology, University Medical Centre Groningen

Like any other biomaterial, implanted collagen scaffolds induce a series of damage-inflicted processes that include wound healing, inflammation and the foreign body reaction (FBR). Macrophages play a pivotal role in the tissue response. These macrophages interrogate the biomaterial surface, release proteolytic enzymes and/or phagocytose the biomaterial. Under certain circumstances, the macrophages may fuse, to form multinucleated foreign body giant cells. Collagen-based biomaterials can be cross-linked to enhance the stiffness and to dampen the rate of biological degradation. In addition, non-crosslinked (native) collagen as well as denatured collagen, i.e. gelatin, can be used. It is the specific application that determines the choice of the biomaterial. Biomaterial applications must take the tissue response towards biomaterials into account.

Despite the frequent use of collagen-based scaffolds in tissue engineering, remarkably little is known about the nature of the foreign body reaction and the molecular mechanisms that are involved in the breakdown of the scaffolds. In a series of experiments, we observed that the tissue response towards the gelatin disks and the (non-)crosslinked, native collagen disks differs markedly with respect to the number of macrophages, the efficiency of giant cell formation, the size of the giant cells, the influx of neutrophils, and the micro-environment (presence of IL-13 and TIMP-1), the expression of MMPs and cathepsin K, and the expression of the collagen receptors Endo180 and DDR-2. Thus, the physical state of the collagen itself (denatured or native) as well as its chemical nature (type of cross-link) has a dramatic impact on the outcome of the foreign body reaction. We will discuss the observed findings in terms of degradation rates of the scaffolds and the mechanisms involved in this degradation. In addition, we will show that macrophages inside and outside the biomaterial have different phenotypic properties.

Keywords. collagen, macrophages, foreign body reaction, degradation

(6.O1) GENE EXPRESSION PATTERNS IN OSTEOGENIC CELLS TREATED WITH STRONTIUM-SUBSTITUTED BIOACTIVE GLASSES

Gentleman E (1), Autefage H (1), Park G (1), Stevens MM (1)

1. Imperial College London, Department of Materials and Institute of Biomedical Engineering

Introduction. Bioactive glasses (BG) are used as bone replacements because they bond with living tissue and dissolve upon implantation, releasing ions that stimulate bone formation. Strontium (Sr) ranelate is an anti-osteoporosis drug that works via Sr cations, which stimulate osteoblast differentiation and prevent osteoclast-mediated bone resorption. We have previously shown that BG in which Ca was substituted with Sr, promote osteoblast proliferation and activity and decrease osteoclast activity and resorption. Here, we examine the effects of Sr-substituted BG on gene expression patterns in cultures of human mesenchymal stem cells (hMSC) and primary osteoblasts (hOB).

Methods. BG in which 0, 10 or 100% of Ca was substituted with Sr were produced. Culture media was created by soaking with BG particles to release their active ions. hMSC and hOB from 3 separate donors were treated with BG-treated media for up to 14 days. RNA was isolated and gene expression patterns were analysed by quantitative real-time RT-PCR and whole genome microarray.

Results and Discussion. We demonstrate that genes for bone-specific transcription factors and proteins are upregulated in cultures treated with BG compared to controls treated with basal medium. In osteoporosis patients treated with Sr ranelate, an anabolic effect on bone formation has been observed. Here, we show that osteogenic genes are upregulated to a greater extent in hMSC and hOB treated with Sr-substituted BG compared to standard all Ca BG controls. Taken together, these results suggest that Sr-substituted BG upregulate key genes in bone development, suggesting that it may be possible to reproduce the anabolic effect on the skeleton produced by orally delivered Sr ranelate in a biomaterial that releases Sr locally. More extensive data analysis of microarray results may also reveal insights into the mechanism by which Sr acts on osteogenic cells.

Keywords. Bioactive glass, strontium, bone regeneration.

(6.O2) THE ROLE OF HYDROLYTIC ENZYMES AND REACTIVE OXYGEN SPECIES IN AN IN VITRO MODEL OF MACROPHAGE-MEDIATED DEGRADATION OF POLY(TRIMETHYLENE CARBONATE) NETWORK FILMS.

van Kooten TG (1), Bat E (2), Kuijer R (1), Grijpma DW (3)

1. UMCG Groningen, Department of Biomedical Engineering, The Netherlands; 2. University of Twente, Molecular NanoFabrication Group, The Netherlands; 3. University of Twente, Department of Biomaterials Science and Technology, The Netherlands.

Resorbable polymers are used in the human body as drug carriers, as scaffolds for tissue regeneration, and in preparation of degradable implants such as sutures. Macrophages play an important role in the degradation of

these polymers. Enzymes and reactive oxygen species (ROS) were shown to be involved in the degradation process. This research aims at elucidating the involvement of enzymes and/or ROS in the degradation mechanism of gamma irradiated poly(trimethylene carbonate) (PTMC) films.

The roles of enzymes and ROS in degradation were evaluated by culturing murine J774 macrophages on PTMC network films containing inhibitors for specific degradation pathways. The influence of complement on the degradation process was assessed as well. Degradation was quantified by determining mass loss of the PTMC discs. Macrophage activity was measured through cytokine release of Il-6, MCP-1 and MIP-1α as determined with ELISA. Cell coverage was calculated from images obtained with confocal microscopy.

Cholesterol esterase was found to be the main contributor to degradation as assessed by the inhibition of degradation by diethyl umbelliferyl phosphate. The results furthermore demonstrated that acid proteases (inhibited by pepstatin A), serine and cysteine proteases (inhibited by phenylmethyl sulfonyl fluoride) and ROS (indirectly inhibited by apocynin through NADPH oxidase and nitric oxide synthase) contribute less to the degradation of PTMC networks. Activity of macrophages was high both with and without the influence of inhibitors, as indicated by high concentrations of secreted cytokines MCP-1 and MIP-1α. Degradation in media without complement was higher than in media with complement.

The presented macrophage culture model is helpful in reducing the number of animal experiments and provides a useful fast, in vitro model to investigate the mechanism of in vivo degradation of biodegradable polymers.

Keywords. macrophage, model, biodegradation, pTMC, degradable

(6.O3) INNOVATIVE IN-VITRO POLYCULTURE MODEL, AS AN ARTIFICIAL LIVING PERITONEUM, FOR ABDOMINAL MESH EVALUATION

Lefranc O (1), Vernier A (1), Barrier F (1), David L (2), Frank L (2), Siali R (2)

1. Covidien; 2. Therapol

Introduction. In-vitro assays are unavoidable in the medical device evaluation process, and often represent the first step for biomaterial characterization. However, it is today well accepted that in-vitro cell culture assays are non relevant of real life conditions.

Materials and Methods. In this study, a coculture model involving the cells present in a healthy peritoneum was developed. Human fibroblasts, macrophages, mesothelial cells and/or endothelial cells were seeded in a type I collagen matrix and cocultured up to reach a stable living structure. The model showed baseline cytokine expression which was dramatically increased when a wound was induced on the surface.

Polypropylene (PP), Polyester (PET) and collagen coated polyester (PETc) prostheses, presenting increasing hydrophilicity gradients, were deposited on the coculture model to evaluate the model different reactions when in contact with those materials.

Results and discussion. To generate a wound, scalpel cuts were applied on the coculture model inducing dramatic pro-inflammatory cytokine secretion.

As already observed under single cell culture, the coculture models still showed better cell adhesion and proliferation on prostheses following hydrophilicity gradients (PET and PETc) when compared with hydrophobic prostheses (PP).

Furthermore, the tri-cells models presented a measurable shrinkage (30%* in surface, *p<0,05) as reaction to the bare prosthetic materials (PP and PET) while no model shrinkage was measured at all for the collagen coated prostheses (PETc), showing the collagen benefit for the device integration. No shrinkage at all could be measured when endothelial cells were added to the coculture, highlighting physiological contractile reactions.

Conclusion. A new in-vitro complex coculture model was developed as a living peritoneum. This model presented better cell compatibility correlated with surface hydrophilicity gradients and highlighted the collagen positive impact on the in-vivo integration reaction.

Keywords. in-vitro, inflammation, mesh

(6.O4) HYDROPHILIC RESORBABLE AND BIOCOMPATIBLE POLYMER SYSTEMS AS BIOACTIVE COATINGS OF POLYPROPYLENE MESH AND CONTROLLED RELEASE OF ANTIBIOTICS FOR TISSUE INTEGRATION

Fernández-Gutiérrez M (1), Olivares E (2), Bellón JM (2), San Román J (1)

1. Institute of Polymers, CSIC. Juan de la Cierva 3, 28006 – Madrid, Spain CIBER-BBN; 2. University of Alcala de Henares, Spain CIBER-BBN

Introduction. The reparative process of hernia defects are in general based on the apposition of polypropylene meshes as biostable implants, which guarantees the biomechanical stability of the affected tissue or organ. After more than 30 years of clinical application, it is clear that one critical point is the infection of the tissues or organs in contact with the mesh and the consequences of the infection process reaching statistical values around 20% in a period of 2 or 3 months after implantation, depending on microorganism strain origin of the infection process. In this work we present a novel an excellent results on the application of bioactive and resorbable hydrogel polymers based on copolymers of hydroxyethyl methacrylate HEMA and 2-acrylamide- 2-methyl propanesulfonic acid AMPS, as bioactive coating of lightweight polypropylene PP meshes, and the addition to the polymer system of a well known antibiotic, vancomycin at a concentration of 20 wt-% respect to the coating of the polymer applied.

Materials and Methods. The coating of the PP mesh was obtained by the deeping of the mesh in a solution of 10 % of copolymer with a composition 20 mole-% of AMPS and 80 mole-% of HEMA containing 20% of vancomycin. After drying a homogeneous coating of 2.0 µm was obtained.

The antibacterial activity of the coated meshes was tested by analyzing the inhibition areas of proliferation of agar plates inoculated with Staphylococcus aureus SA or S.epidermidis SE. The bioactivity was analyzed in vitro using fibroblast cultures, and in vivo by implantation of coated meshes in infected areas of the dorsal muscle of