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In-vivo usage of biomaterials: A snapshot of current activity

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ABSTRACT

The global market for end-use medical devices is worth around $50 billion of which the materials market, from which the devices are manufactured, has annual sales of approximately $1 billion. The key objective in selecting materials for a particular end-use application is to ensure that the resultant device has comparable properties to the tissue that it is replacing and does not induce any adverse reaction. In this context the term device is used to mean implants or tissue repairs e.g. intervertebral discs, tissue scaffolds and repairs to bone defects. Some of these applications use materials that degrade either *in vivo* or *in vitro* over periods of time that range from days to years. The rate of degradation depends on the chemistry of the material, its location and very importantly, its structure. This report provides a snapshot view of the materials that are being used or considered for use as substitutes for bone, cartilage, intervertebral discs and skin.

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Approved on behalf of Managing Director,
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1 INTRODUCTION

The global market for biocompatible end-use devices is around $50 billion with current annual sales of materials of approximately $1 billion. Estimates of the growth rate of this market ranges from 10% to 15% with sectors such as drug delivery, urology, artificial tissue and tissue engineering expected to experience higher growth rates of 16% to 28%. End-use devices include implants, valves, bone repair, grafts, pacemakers, dental materials, drug delivery systems, dialysis/separation/filtration systems, catheters, stents and artificial organs. These devices are manufactured from a spectrum of materials that include metals, synthetic and natural polymers, ceramics, pyrolytic carbon and composites. Biocompatibility is a key issue; new developments in biotechnology, medical prostheses and tissue engineering depend on the interactions between cells and materials. In some cases cell adhesion needs to be discouraged, e.g. in urinary catheters whilst in others cell adhesion is essential e.g. in developing tissue engineered medical products.

The prospect of being able to generate new tissue using the patients own cells to repair existing damaged tissue has stimulated an increased amount of research activity in biocompatible materials. During the past three years there has been strong growth in the number of papers published in the open literature in tissue engineering as shown in Figure 1. The increased number of publications over the past decade is in itself an unreliable indicator of market activity. However as Figure 2 shows the trend in published papers is mirrored by a similar increase in the number of patents in tissue engineering. Registrations are dominated by the USA which is recognised as the market leader.

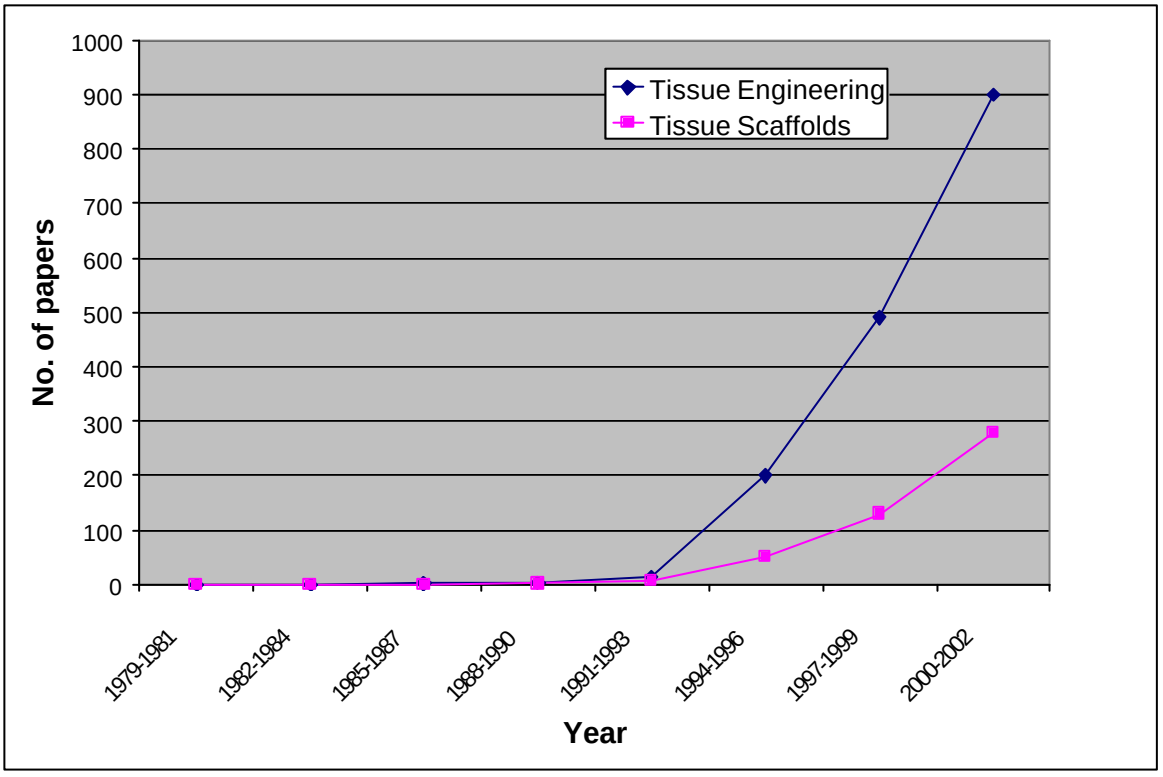

Figure 1 The number of published papers in tissue engineering as a function of time over the past two decades.

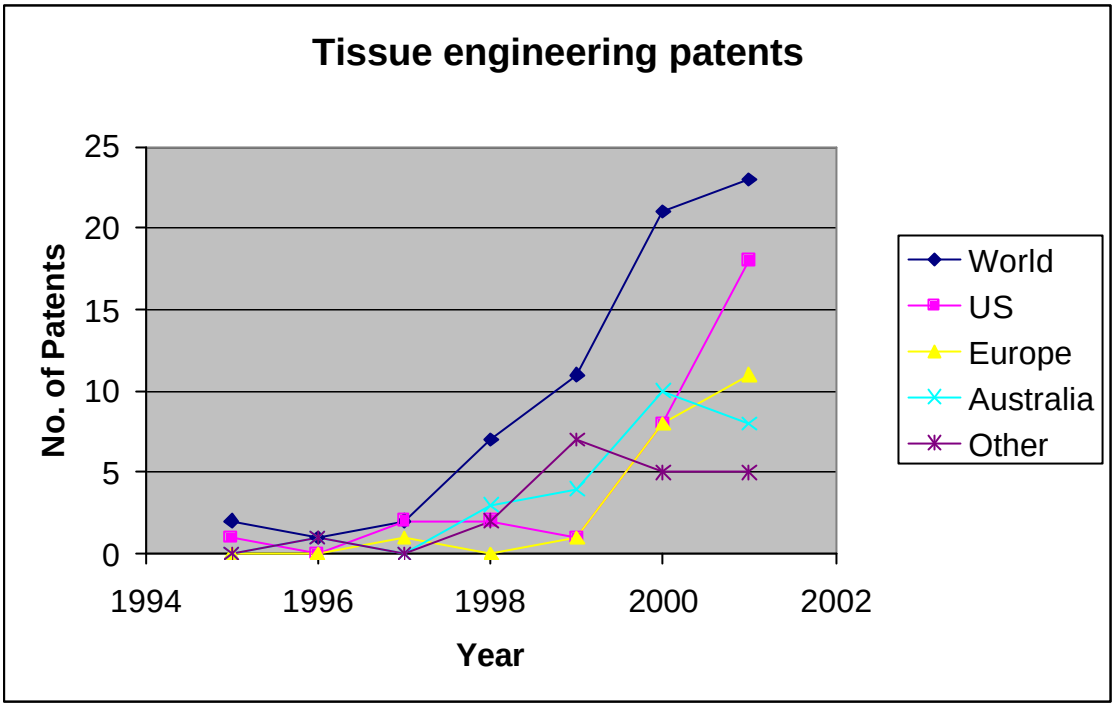

Figure 2 The number of patents containing the term 'tissue engineering' published as a function of time during the past decade.

This report provides a snapshot view of the materials that are currently being assessed for their suitability as tissue scaffolds or as end-use devices in laboratory animals. The search was carried out using the Web of Science [1] and the National Library of Medicine [2] and covers the past twenty years. Clearly the literature for *in vivo* studies of biomaterials is colossal and well beyond the scope of this report. We have chosen to focus on four areas: **bone and bone substitutes**, **cartilage**, **intervertebral discs** and **skin repair templates**, which form the major areas of tissue engineering and have products in the market or close to market. The goal of the literature search has not been to produce a considered view of which materials, coatings, structures, surgical procedures, cell types or animal model is best for a particular application but to provide a horizontal view of which materials are being used in what kind of application. The strength of this approach is to provide a standoff assessment of a very detailed and extensive literature.

Literature searches on each of these areas were carried out using combinations of the keywords listed under each heading in Table 1. The references generated are presented in tabular form (Annex 1-4) that list the animal model, the material used, the location of the implant and publication details. Recent reviews of the literature in each of the specific areas are listed in Annex 5.

Table 1 Keywords used in searching 'The Web of Science' and the National Library of Medicine PUBMED databases.

Bone and Bone substitutes	Cartilage	Intervertebral discs	Skin repair
Bone	Cartilage	Spine	Skin
Substitutes	Degradation	Substitutes	Substitutes
Cements	*In-vivo*	*In-vivo*	Grafts
Degradation	Animal testing	Animal testing	Animal testing
In-vivo	Implant	Replacement	Animal models
Animal testing		Intervertebral disc	*In-vivo*
Replacement			Degradation
Implant			Templates
			Implants

2 BONE AND BONE SUBSTITUTES

Despite its apparent inertness bone is continually remodelling itself according to how much load it is placed under and the age of the individual; the effects of osteoporosis. The ability of bone to respond to changing loads, whilst an asset for the body, has bng been recognised as having an adverse effect on the long-term performance of implants. Lack of osteointegration (or osseointegration) and poor load transfer to the surrounding bone sheath are two of the most important factors responsible for bone atrophy and subsequent loosening of the implant. A significant amount of research has been carried out in this area to overcome these issues that focuses on fostering osteointegration. Osteointegration can be achieved in a number of ways:

- Providing a rough surface finish to provide a keyed surface (this increases the contact area with bone, improving load transfer but is not osteointegration).
- Coating metallic implants with osteoconductive porous coatings.
- Using bone derived from allografts, autografts or xenografts.
- Seeding polymer or ceramic scaffolds with, for example, osteoblasts or stem cells.
- Using bone-like biocompatible substitutes with comparable mechanical properties e.g. coralline materials.

Osteointegration is typically assessed through histological examination. The references listed in Annex 1 provide examples of these avenues of research. The materials that are used reflect the mechanical needs of the repair, for example, titanium is traditionally used to repair failed high-load bearing hip joints, whereas low-load bearing areas e.g. cranial defects can be repaired using polymer-based materials.

A significant body of literature exists that explores the use of allografts, autographs and xenografts for repairing bone. This route to repair has been excluded from this survey, which instead focuses on

the use of substitute materials, although some demineralised bone publications have been included, especially where this material has been used as a composite. The materials that are being considered for use as bone substitutes include:

- Synthetic polymers
    - Polycaprolactone
    - Poly (lactide-co-glycolide) (PLGA)
    - Poly(etheretherketone) PEEK
- Natural polymers
    - Collagen
- Bone-like materials
    - Corals
    - Wood
    - Hydroxyapatite-tricalcium ceramics
    - Bioglasses
- Metals
    - Titanium with controlled surface roughness or with coatings
- Composites
    - Collagen/bone particles
    - Ceramic/bone-particles

These materials with the exception of titanium itself are commonly doped with recombinant proteins that encourage cell growth such as human osteogenic protein-1 and 2 (rhOP-1 and rhOP-2).

Some of the bone substitute materials e.g. titanium remain unchanged in the *in vivo* environment whilst others e.g. demineralised bone, coralline materials and ceramics will be slowly and continuously remodelled on the timescale of natural bone after osteointegration. Materials such as collagen or PLGA that are used as temporary scaffolds to accommodate cells and act as reservoirs for growth factors will by definition degrade over a relatively short period of time. The rate of degradation depends on the location of the implant, its physical structure and on the materials chemistry. For example, the degradation behaviour of PLGA can be changed from days to years depending on the proportions of lactide to glycolic acid in the copolymer.

3 CARTILAGE

Cartilage is used in the body to provide articulating surfaces in joints such as the knee or to provide the key structure element in relatively flexible structures such as the nose and ears. Mechanically, cartilage needs to be creep resistant and able to tolerate a certain amount of shock loading, a property that is especially important for the articulating surfaces of joints e.g. femoral head / acetabulum. The ability of cartilage to tolerate sudden load changes is due to a combination of viscoelasticity and fluid flow, a mechanism that is now being mimicked in the design of the soles of sports footwear.

Examples of materials that are under consideration for producing cartilage are based on either:

- Synthetic polymers e.g.
  - Poly(caprolactone) (PCL)
  - Poly(glycolic acid) (PGA)
  - Poly(DL lactide-co-glycolide) (PLGA)
  - Poly(ethylene glycol) (PEG)
  - Polyurethane (PU)
  - Poly(phenylene oxide) (PPO)
  - Polyethylene (PE)
- Natural polymers e.g.
  - Collagen
  - Alginates
  - Agarose (polymeric carbohydrate)
  - Gelatine
  - Hyaluronic acid

These materials are either used as gels (e.g. alginates, agarose) or as porous foams (e.g. polyethylene, polycaprolactone). Both the naturally occurring polymers and most of the synthetic materials degrade *in vivo* over timescales that reflect both the location of the implant, its physical structure and the materials chemistry. The mechanical properties of cartilage scaffolds can be controlled by varying the cross-link density of gels, the pore volume of foams or by chemical means e.g. the proportion of hard to soft segments in polyurethanes to suit specific needs. Artificial cartilage is often manufactured *in vitro* using a scaffold that degrades to leave a block of viable cartilage that can be implanted. In this type of application the degradation kinetics of the material obviously play a key role and need to be matched to the integration of the maturing cell colony to form a block of tissue. The need for chondrocytes to be mechanically stimulated in order to maintain their phenotype must also be taken into consideration in designing the time dependent properties of the scaffolds. Establishing the boundaries for mechanical stimulation has been explored using *in vitro* methods culturing cartilage in mechanically active bioreactors and *in vivo* using ectopic sites.

Much of the published literature concerned with producing cartilage focus on healing damaged areas in situ based on drug delivery systems. During the past three to four years a number of approaches in the treatment and regeneration of damaged cartilage have been used which include genetically modified proteins such as injecting modified growth factors at the required site, using recombinant proteins, or using gene therapy to deliver genes into live tissues using either virus-mediated gene delivery or direct injection of plasmid DNA.

4 INTERVERTEBRAL DISC

Damage to intervertebral discs can, in some cases, be repaired using drugs to relieve pressure on the disc thus allowing it to regenerate. For more extensive damage the course of action is usually excision of the disc followed by spinal fusion. This process both adversely affects mobility and can, by altering the normal distribution of loads, place unacceptable levels of stress on adjacent joints. These limitations are responsible for research into the development of artificial intervertebral discs

that have comparable mechanical properties to the native materials. Substitution of the natural disc with an artificial substitute should, in principle, have no adverse influence on back mobility or on the loads applied to adjacent discs.

Spinal fusion usually relies on autologous bone harvested from the patient to fill the space previously occupied by the intervertebral disc. Harvesting and preparing autologous bone places an additional burden on the patient and adds to the cost of performing the operation. Substituting glass-ceramics or porous hydroxyapatite doped with growth factors for autologous bone offers a potential route to reducing these operational costs. A number of reviews have been published that describe the current state of research into artificial intervertebral discs (Annex 5). The principle of the artificial disc is to substitute the natural disc with an elastomeric construction that ideally has comparable viscoelastic performance to the natural material. This 'viscoelastic unit' is contained between metal plates (Charite disc) or enclosed within a three-dimensional mesh produced from a material such as ultra high molecular weight polyethylene fibres.

The materials used for the viscoelastic cores include:

- Polycarbonate-urethane thermoplastic elastomer
- Hydrogel (identity commercially sensitive)
- Polyurethane (PU)
- Polydimethylsiloxane (PDMS)

These viscoelastic cores are typically housed between porous ceramic-coated titanium plates, but woven meshes are also being experimentally investigated. These are typically manufactured from polyethylene. An alternative approach to disc substitution is to use tissue engineering to produce replacement tissue and some work is being carried out in this area using atelocollagen gel to house annulus fibrosus cells. Materials that are used to pack the intervertebral gap after a discectomy in spinal fusion operations include:

- Titanium cages
- Hydroxyapatite
- Zirconium oxide
- Bioactive glass ceramics (BAS-O ™)

5 SKIN REPAIR TEMPLATES

Skin grafts or other skin replacement procedures are used to repair deep second degree or full thickness third degree burns where both the epidermis and dermis have been irreparably damaged. Lack of sufficient donor sites on burn patients and concerns over the safety of skin grafts has stimulated a search for skin substitutes that can act as temporary or permanent skin replacements. A key issue in the repair of severe burns, particularly where the patient has incurred widespread skin damage is to quickly stem fluid loss and to inhibit microbial infection. Skin templates can be obtained from skin harvested from cadavers. This material is treated to produce an acellular scaffold that acts as a housing for dermal re-growth. 'Synthetic' skin substitutes are commercially available. These typically consist of two layers, an outer 'waterproof' shell and an inner scaffold usually based on collagen that is doped with either cells or a concoction of growth factors. The purpose of the inner

layer is to stimulate re-growth of the dermis. Once this has been achieved a thin epidermal layer can be substituted for the outer membrane to complete the repair.

The materials used to produce skin templates are based on either:

- Synthetic polymers
  - Poly(caprolactone) (PCL)
  - Polyester/polypropylene
  - Poly(vinyl alcohol) (PVA)
  - Silicone
  - PLGA and PLA, PLLA
  - PTFE (Polytetrafluoroethylene)
  - Ethylene Vinyl Acetate (EVA)
  - Polyaniline (PA)
  - Polyethylene (PE)
  - Polyether
  - Nylon
- Natural polymers
  - Collagen
  - Chitosan
  - Fibrin gel
  - Alginates
  - Hyaluronic acid
  - Gelatine

Both natural and synthetic polymers are used to provide mechanical support for hydrogels seeded with keratinocytes or fibroblasts to form the skin substitute. Examples of this type of application using synthetic polymers include PTFE foam and woven polyester mesh. Porcine intestine and bladder mucosa provide readily accessible natural sources of biodegradable acellular dermal matrix.

6 SUMMARY

This snapshot of how and which materials are being used or explored for use in end-use medical devices reveals a relatively limited palette. The reasons for this are no doubt complex and probably reflect a degree of conservatism within the medical community coupled with the need to reduce the time to market by utilising materials that have been accepted by regulatory bodies.

Titanium is the preferred metal for medical applications and is used in areas that are subject to high loads, e.g. artificial joints, dental implants and stents. Natural polymers, especially collagen are used as hydrogels to provide temporary housings for cells and bioactive compounds such as growth factors. Acellular matrices based on collagen are being harvested from cadavers and animal sources (pigs) to provide temporary structural supports for hydrogels in applications that include skin substitutes and bone repair. Naturally occurring porous materials e.g. coral and demineralised bone sourced from cadavers or animal sources provide an alternative to autografts used for bone repair in areas of the body that are subject to load. These materials are usually filled with hydrogels doped with a cocktail of growth factors. There are potential safety issues in using collagen-based matrices

obtained from cadavers or animals from prions and other pathogens that may not have yet been identified. Synthetic polymers such as PLGA and PCL are used throughout the body in a wide range of applications including sutures and as tissue scaffolds. PLGA has an additional advantage in that the copolymer can, in principle, be tailored to degrade over a specific timescale by changing the proportions of the constituents although in practice the degradation rate is strongly influenced by the structure of the construct and its location. Other synthetic polymers are more resistant to degradation through hydrolysis or by enzyme attack especially materials such as polyethylene that are known for their inert properties.

This snapshot view of the biomaterials literature has revealed how rare it is for publications in this field to quote the material properties in detail, for example, the molecular weight distribution of polymers (the degree of polydispersity), levels of side–branching, degree of crystallinity or the presence of processing aids and how these may change due to sterilization. All of these factors can potentially influence one or more properties of the polymer implant over time in terms of cell adhesion, degradation behaviour and potentially mechanical performance and can have a significant influence on the consistency of manufactured constructs, especially those produced under laboratory conditions.

7 REFERENCES

[1] *The Web of Science* ***http://www.isinet.com/products/citation/wos/***

[2] *National Library of Medicine PubMed* ***http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed***

8 ACKNOWLEDGEMENTS

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ANNEX 1 – *IN VIVO* STUDIES OF BONE AND BONE SUBSTITUTE MATERIALS

Title:	Repair of calvarial defects with customised tissue-engineered bone grafts II. Evaluation of cellular efficiency and efficacy *in vivo*			**Author(s)/ Reference:**	Schantz JT, et al., Tissue Eng., 2003, 9, S127-S139
Material:	Polycaprolactone (PCL) scaffold with osteoblasts and mesenchymal progenitor cells	**Animal Model:**	Rabbits	**Implant Location:**	Calvarial defects
Summary:	Osteoblasts and mesenchymal progenitor cells were seeded in combination with a fibrin glue suspension into 40 PCL scaffolds. The scaffolds were fabricated with a precise microarchitecture using rapid prototyping. These were implanted into 15mm diameter calvarial defects created in rabbits. Early bone formation was observed with X-ray radiographs. Mechanical testing highlighted the level of integration with the surrounding bone. The amount of calcification was measured using computer tomography				
Title:	*In vivo* efficacy of bone-marrow-coated polycaprolactone scaffolds for the reconstruction of orbital defects in the pig			**Author(s)/ Reference:**	Rohner D, et al., J. Biomed. Mater. Res. Part B, 2003, 66B(2), 574-580
Material:	Bone marrow coated polycaprolactone (PCL) scaffolds	**Animal Model:**	Pigs	**Implant Location:**	Orbital defects
Summary:	Fused deposition modelling was used to manufacture polyacprolactone (PCL) scaffolds. These were coated in bone marrow and placed into surgically created defects in each orbit of eight pigs. It was shown via histological and histomorphometric analyses that there was 14.1% new bone formation				
Title:	Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect			**Author(s)/ Reference:**	Arinzeh TL, et al., J. Bone & Joint Surgery - American Volume, 2003, 85A(10), 1927-1937
Material:	Allogeneic mesenchymal stem cells loaded onto a hollow ceramic cylinder consisting of hydroxyapatite-tricalcium phosphate	**Animal Model:**	Dogs	**Implant Location:**	Femoral diaphysis
Summary:	A critical-sized bone defect was created in the femoral diaphys of 12 dogs. Each defect was treated with allogenic mesenchymal stem cells loaded onto a hollow ceramic cylinder consisting of hydroxyapatite-tricalcium phosphate.Techniques used to evaluate the success of the implant included histology and radiography. The results showed that the repair of the defects was enhanced by the implant and no adverse immune response was detected				
Title:	Bone-bonding ability of anodic oxidized titanium			**Author(s)/ Reference:**	Liang et al., Bioceramics, Vol. 240-2, 2003, 923-926
Material:	Anodic oxidised bioactive titanium	**Animal Model:**	Mature rabbits	**Implant Location:**	Anodic oxidised bioactive titanium
Summary:	Anodic oxidized bioactive titanium plates were prepared in 1M H2SO4 at 155v DC. These were implanted into the proximal metaphyses of the tibiae of mature rabbits. Higher bone-bonding ability of these implants was observed at early stage of implantation. After detaching test, no titanium processing layer breakage was observed histologically. SEM images of bone-implant interface showed direct bond without soft tissue layer.				

Title:	Juniper wood as a possible implant material			**Author(s)/ Reference:**	Gross et al., J. Biomedical Materials Research Part A, 64A, 2003, 672-683
Material:	Juniperus communis wood	**Animal Model:**	Rabbits	**Implant Location:**	Femoral bone
Summary:	Juniperus communis wood is dense, durable and strong and has naturally impregnated essential oils. This study investigated the toxicity of the oil (examined orally and by intravenous injection), the effect of sterilization on the mechanical properties of the wood (with boiling water) and bone attachment in rabbits. Wood shaped into the form of femoral implants was implanted into rabbits and displayed good acceptance by the body.				
Title:	*In vivo* bone engineering in a rabbit femur			**Author(s)/ Reference:**	Fialkov et al., J.Craniofacial Surgery 4 (3), 2003, 324-332
Material:	Poly(lactide-co-glycolide) (PLGA)	**Animal Model:**	Rabbit	**Implant Location:**	Femoral bone defect reconstruction
Summary:	Poly(lactide-co-glycolide) (PLGA) scaffolds seeded with marrow-derived progenitor cells were evaluated for bone repair in femoral critical-sized defects in rabbits. Significant bone formation was measured using radiography and histology.				
Title:	An initial investigation of photocurable three-dimensional lactic acid based scaffolds in a critical-sized cranial defect			**Author(s)/ Reference:**	Burdick et al., Biomaterials 24 (9), 2003, 1613-1620
Material:	Poly(lactide-co-glycolide) (PLGA)	**Animal Model:**	Rat	**Implant Location:**	Cranial defect
Summary:	A porous poly(lactide-co-glycolide) (PLGA) scaffold fabricated with 80% porosity and pore diameters ranging from 45 to 150 mm were implanted in a critical-sized cranial defect in rats. The scaffolds degraded in 8 months and possessed an elastic modulus similar to that of trabecular bone				
Title:	Use of injectable calcium-phosphate cement for the fixation of titanium implants: an experimental study in goats			**Author(s)/ Reference:**	Jansen et al., J. Biomedical Materials Research Part B – Applied Biomaterials. 66B, 2003, 447-456
Material:	Fixation of titanium implants using injectable calcium phosphate cement	**Animal Model:**	Goat	**Implant Location:**	Trabecular bone of the medial femoral condyle
Summary:	This study examined the fixation of two types of titanium implants with the use of injectable calcium-phosphate cement. The implants were placed in trabecular bone of the medial femoral condyle of goats. On the basis of observations, it was concluded that the use of this cement might facilitate earlier loading of press-fit titanium implants				
Title:	Induction of ectopic bone formation by using human periosteal cells in combination with a novel scaffold technology			**Author(s)/ Reference:**	Schantz JT, et al., Cell Transplantation, 2002, 11(2), 125-138.
Material:	Polycaprolactone (PCL) scaffold with human calvarial periosteum cells	**Animal Model:**	Mice	**Implant Location:**	Ectopic site

Summary:	A novel scaffold architecture made of polycaprolactone (PCL) was seeded with human calvarial periosteum cells and implanted into mice. Three-dimensional cell proliferation was observed after 2 weeks and endochondral bone formation with osteoid production was detectable via von Kossa and Osteocalcin staining after 6 and 17 weeks. Histology and SEM revealed that the entire scaffold/bone grafts were penetrated by a vascular network				
Title:	Hard tissue remodelling using biofabricated coralline biomaterials			**Author(s)/ Reference:**	Vago R, et al., J. Biochemical and Biophysical Methods, 2002, 50(2-3), 253-259.
Material:	Hydrocorals hydrozoan, M. dichotoma	**Animal Model:**	Rabbits	**Implant Location:**	Defect in medial femoral condyles
Summary:	This study examined the potential uses of biofabricated marine carbonate materials as biomatrices for remodelling bone and cartilage tissue. The rate at which bone ingrowth occurs depends on many factors, including pore size and the interconnectivity of the implanted structure. Grafting into osteochondral defects in rabbits demonstrated that this material is highly biocompatible with cartilage and bone tissue. After 4 months, the biomaterial had been replaced by new tissue.				
Title:	Application of an X-ray microscopy technique to evaluate tissue-engineered bone-scaffold constructs			**Author(s)/ Reference:**	Schantz et al., Materials Science and Engineering C, 20, 2002, 9-17
Material:	Polycaprolactone-hydroxyapatite blend (90:10 wt.%)	**Animal Model:**	Nude mice	**Implant Location:**	Next to spine
Summary:	The aim of this study was to evaluate the feasibility of X-ray microscopy technique to analyse tissue-engineered samples. 3-D scaffolds were made of polycaprolactone (PCL)-hydroxylapatite (HA) (90/10 wt.%). A primary explant system of the morcellized grafts with cells from human calvarial corticocancellous bone was established within the PCL-HA constructs. Tissue constructs were cultured in vitro for 3 weeks and then implanted into the back of Balb C nude mice. Grafts were explanted after 17 weeks and tissue formation was assessed via XSAM and CT scan and histology.				
Title:	Testing bone substitutes in a small animal model of revision arthroplasty			**Author(s)/ Reference:**	Clarke S et al., Journal of Materials Science – Materials in Medicine, 13, 2002, 829-836
Material:	Beta-tricalcium phosphate with Gelform® which is moulded around proximal end of either an uncoated stainless steel pin or pin coated with hydroxyapatite	**Animal Model:**	Rats	**Implant Location:**	Pin with ceramic collar implanted into proximal tibia
Summary:	This study evaluated a modification of the rat-pin model to enable testing of bone substitute materials. A defect was created around a stainless steel implant in the proximal tibia of a rat. This defect was filled with beta-tricalcium phosphate (TCP). Large particles (90-312mum) of TCP were mixed with Gelfoam(R) to form a paste which was then molded around the proximal end of the stainless steel pin coated or uncoated with hydroxyapatite (HA). The pin with its ceramic collar was then implanted into the proximal tibia of rats. Longitudinal sections of each tibia were stained with toluidine blue and labelled for tartrate resistant acid phosphatase (TRAP). The remodelling process peaked at 3 weeks around the HA coated pins and at 6 weeks around the uncoated implants. There was considerable residual betaTCP present, which was well tolerated as the particles were often encased in bone.				
Title:	The use of a coin shaped implant for direct in situ measurement of attachment strength for osseointegrating biomaterial surfaces			**Author(s)/ Reference:**	Ronold H et al., Biomaterials, 23, 2002, 2201-2209

Material:	Grade 2 titanium (ASTM B 348) test surfaces blasted with titanium dioxide (TiO_2) particles. Polytetrafluoroethylene (PTFE) caps were introduced to resist bone growth towards the vertical faces of the implant as well as bone overgrowth.	**Animal Model:**	Rabbit	**Implant Location:**	Tibia
Summary:	A flat coin shaped titanium implant was placed onto the cortical bone of rabbit tibia without mechanical fixation to the bone.				
Title:	Histomorphological, histomorphometrical and biomechanical analysis of ceramic bone substitutes in a weight-bearing animal model			**Author(s)/ Reference:**	Kessler S et al., Journal of Materials Science: Materials in Medicine, 13(2), 2002, 191-195
Material:	Alpha tricalcium phosphate, neutralised glass ceramic and a composite material of its copolymer	**Animal Model:**	Sheep	**Implant Location:**	Medial tibial head, wedge-shaped defects
Summary:	The biomechanical properties, the osteoconductive capacity and the degradation rate of alpha tricalcium phosphate (alpha TCP), a neutralized glass ceramics (GB9N) and a composite material (GB9N+copolymers) were studied. Substitutes were implanted in the medial tibial head of the right lower leg of sheep in a standardized surgical technique. After nine months the implants were harvested and prepared for histomorphological and histomorphometrical investigations (undecalcified Masson Goldner staining). No significant differences for the maximum fracture load as well as for the yield strength were detected between harvested specimens and bone blocks from the contralateral tibia. All substitutes showed osteoconduction, leading to a continuous ingrowth of new formed bone. However in the composite material soft tissue could be identified within the scaffold and there were signs of ongoing bone remodelling, nine months after implantation.				
Title:	Electromagnetic stimulation on the bone growth using backscattered electron imaging			**Author(s)/ Reference:**	Ottani V, et al., Micron, 33(2), 2002, 121-125.
Material:	Hydroxyapatite (natural and synthetic)	**Animal Model:**	Rabbits	**Implant Location:**	Tibia
Summary:	Natural (NA) and synthetic hydroxyapatite (HA) with a grain size <50um were implanted in rabbit tibiae to examine the effect of electromagnetic stimulation (PEMF). Bone samples were processed for LM, TEM and SEM using a backscatter electron detector for the evaluation of bone growth. The study showed that HA has more osteoconductivity than NA and showed that PEMF-treatment results in accelerating bone formation at early time periods				
Title:	Experimental procedure for the evaluation of the mechanical properties of the bone surrounding dental implants			**Author(s)/ Reference:**	Soncini M et al., Biomaterials, 23(1), 2002, 9-17
Material:	Tioblast™ Fixture (titanium fixture), defect filled with deantigenate bovine bone particles (Bio-Oss®) and covered with a resorbable Polygalactin membrane (Vycril™)	**Animal Model:**	Sheep	**Implant Location:**	Tibia with and without defects
Summary:	This paper focuses on an experimental procedure to evaluate the mechanical properties of the bone surrounding dental implants. Microradiographic analyses on bone fixed with titanium implants in sheep showed that the mechanical properties of the bone surrounding the implant improve, as does the healing time				

Title:	A composite graft material containing bone particles and collagen in osteoinduction in mouse			**Author(s)/ Reference:**	Tsai CH, et al., J. iomedical Materials Research 2002, 63(1), 65-70
Material:	60% collagen and 40% demineralised allogenic bone matrices (DymaGraft (TM)) composite	**Animal Model:**	Mice	**Implant Location:**	Left thigh muscle
Summary:	The goal of this study was to evaluate the characteristics of composite graft implants in the mineralization process in an animal model compared with demineralised freeze-dried bone allograft (DFDBA) powder and pure collagen. Xenogenic collagen isolated from human tendon, skin or bone was added to the bone-graft particles to form a composite sponge-like implant. This material is commercially available and consists of 60% collagen and 40% DFDBA. It was concluded that a higher rate of calcification and bone formation was produced in the composite graft implant compared to the DFDBA implant. The composite graft material (DynaGraft), which contains both collagen and DFDBA proved to more effective for bone formation than particle components alone				
Title:	Use of collagen sponge incorporating transforming growth factor-beta1 to promote bone repair in skull defects in rabbits			**Author(s)/ Reference:**	Ueda H, et al., Biomaterials 2002, 23(4), 1003-1010.
Material:	Collagen sponge incorporating transforming growth factor-beta 1 (TGF-1) with and without label I-125	**Animal Model:**	Rabbits and mice	**Implant Location:**	Skull defects in rabbits and subcutaneously in the backs of mice
Summary:	The objective of this stuffy was to evaluate the potential of collagen sponge incorporating growth transforming growth factor-beta1 (TGF-beta1) to enhance bone repair. The collagen sponge was prepared by freeze-drying aqueous foamed collagen solution. These were implanted into the backs of mice. Via degradation profiles and SEM it was concluded that the collagen sponges were able to release biologically active TGF-beta1 and were a promising material for bone repair				
Title:	Overexpression of the granulocyte colony-stimulating factor gene impairs bone morphogenetic protein responsiveness in mice			**Author(s)/ Reference:**	Kuwabara H, et al., Laboratory Investigation 2001, 81(8), 1133-1141.
Material:	Collagen pellet containing recombinant human bone morphogenetic protein (BMP)-2	**Animal Model:**	Transgenic and normal mice	**Implant Location:**	Ectopic site - dorsal subfascial pocket
Summary:	As a way of determining the role of Granulocyte colony-stimulating factor (G-CSF) in bone formation *in vivo*, an ectopic bone was induced subcutaneously into G-CSF transgenic mice by bone morphogenetic protein (BMP)-2. It was concluded that the permanent expression of G-CSF could retard the differentiation process of osteoblasts by impairing the initial induction of mesenchymal cells.				
Title:	Effect of nickel-titanium shape memory metal alloy on bone formation			**Author(s)/ Reference:**	Kapanen A, et al., Biomaterials, 2001, 22(18), 2475-2480.
Material:	NiTi (54% nickel by weight, 46% titanium by weight, AO/ASIF stainless steel and AO/ASIF Ti–6Al–4V alloy (90% titanium by weight, 6% aluminium by weight, 4% vanadium by weight	**Animal Model:**	Rats	**Implant Location:**	Ectopic site - under the fascia of the latissimus

Summary:	The aim of this study was to determine the biocompatibility of NiTi alloy on bone formation. Comparisons were made between Nitinol (NiTi), stainless steel (Stst) and titanium-aluminium (6%)-vandium (4%) alloy (Ti-6Al-4V) implanted for 8 weeks under the fascia of the latissimus dorsi muscle in rats. Light microscopy, peripheral quantitative computed tomography (pQCT) and digital image analysis were all used. NiTi had good biocompatibility, as its effects on ectopic bone formation are similar to those of Stst.				
Title:	Nanoindentation study of interfaces between calcium phosphate and bone in an animal spinal fusion model			**Author(s)/ Reference:**	Guo LH, et al., J. Biomedical Materials Research 2001, 54(4), 554-559.
Material:	Porous hydroxyapatite/beta-tricalcium phosphate	**Animal Model:**	Rabbits	**Implant Location:**	Spinal fusion
Summary:	In this study, a porous hydroxyapatite (HA)/beta-tricalcium phosphate (beta-TCP) ceramic was tested as a graft material using a rabbit lumbar transverse process (L5-L6) fusion model. Histomorphological observation revealed integration with the host bone by both cancellous bone formation and cartilage formation. Nanoindentation was used to evaluate the mechanical properties of the implants and the newly formed tissues.				
Title:	Radiologic, mechanical, and histologic evaluation of 2 glenoid prosthesis designs in a canine model			**Author(s)/ Reference:**	Wirth MA et al., J. Shoulder and Elbow Surgery, 10(2), 2001, 140-148
Material:	Hylamer	**Animal Model:**	Dogs	**Implant Location:**	Glenoid (shoulder)
Summary:	A new glenoid design aimed at improving fixation outcomes was compared with conventional keeled glenoids in weight-bearing canine shoulders and found to be superior in achieving osseous integration and fixation				
Title:	Biomechanical and histological aspects of fracture healing, stimulated with osteogenic protein-1			**Author(s)/ Reference:**	Blokhuis TJ et al., Biomaterials, 22(7), 2001, 725-730
Material:	AO external fixator, (rhOP-1) and bovine type I collagen matrix in combination with carboxymethylcellulose (CMC)	**Animal Model:**	Goats	**Implant Location:**	Tibia
Summary:	In this study, biomechanical and histological aspects of fracture healing after an injection of osteogenic protein-1 (OP-1) in a fracture gap in the left tibia of goats. The data showed that fracture healing can be accelerated with a single injection of OP-1, eventually resulting in normally healed bone				
Title:	Evaluation of carriers of bone morphogenetic protein for spinal fusion			**Author(s)/ Reference:**	Minamide A et al., Spine 26(8), 2001, 933-939
Material:	Sintered bovine bone True Bone Ceramics, TBC coated with Type I collagen infiltrated with 100 µg of recombinant human bone morphogenetic protein-2 (rhBMP-2), Type I collagen sheet and Type I collagen sheet containing 100 µg of rhBMP-2	**Animal Model:**	Rabbit	**Implant Location:**	posterolateral intertransverse spinal fusion
Summary:					

Title:	Madreporic hydroxyapatite granulates for filling bone defects			**Author(s)/ Reference:**	Muller-Mai C et al., Unfallchirurg, 104(3), 2001, 221-229
Material:	Macrocrystalline madreporic granular hydroxyapatite	**Animal Model:**	Rabbits	**Implant Location:**	Distal femur epiphysis
Summary:	Two macrocrystalline madreporic granular hydroxyapapatite implants of different size range were implanted into the trabecular bone of the distal femur epiphysis of rabbits. Both of the granular materials tested are useful in filling bone defects. A guided tissue regeneration due to partial implant degradation and subsequent bone formation seems to be impossible since the degradation rate of the materials is too low				
Title:	Evaluation of recombinant human bone morphogenetic protein-2 as a bone-graft substitute in a canine segmental defect model			**Author(s)/ Reference:**	Sciadini MF et al., J. of Orthopaedic Research, 18(2), 2000, 289-302
Material:	External fixator and recombinant human bone morphogenetic protein-2 with a collagen sponge carrier	**Animal Model:**	Dogs	**Implant Location:**	Radial
Summary:	A study was performed in dogs to evaluate the dose-response characteristics and effectiveness of recombinant human bone morphogenetic protein-2 (RHBM P-2) with a collagen sponge carrier. Defects were created in dogs - all received autogenous cancellous bone grafts in one defect and a collagen implant in the other. Radiology and histology confirmed that recombinant human bone morphogenetic protein-2 (RHBM P-2) in a collagen carrier has significant osteoinductive activity				
Title:	Osteoconduction in large macroporous hydroxyapatite ceramic implants: evidence for a complementary integration and disintegration mechanism			**Author(s)/ Reference:**	Boyde A, et al., Bone, 1999, 24(6), 579-589.
Material:	Macroporous hydroxyapatite ceramics	**Animal Model:**	Sheep	**Implant Location:**	Tibiae
Summary:	Large, cylindrical implants of a porous calcium phosphate ceramic ("hydroxyapatite" starting material, HAC) were used to replace far greater than critical-sized sections of the midshaft of sheep tibiae. Bone adaptation to the HAC leads to its fracture and the newly generated surfaces are equally osteoconductive. This leads to a self-propagating, self-annealing system in which defects in the HAC are mended by the intercalation of bone				
Title:	Anti-inflammatory properties of triblock siloxane copolymer-blended materials			**Author(s)/ Reference:**	Tang et al, Biomaterials, 20, 1999, 1365-1370
Material:	PVC modified with polydimethysiloxane-polycaprolactone to make silicone-like polymer	**Animal Model:**	Mouse	**Implant Location:**	Peritoneal cavity
Summary:	A series of polycaprolactone-polydimethylsiloxane- polycaprolactone (PDMS-PCL) copolymers were developed. Polyvinyl chloride (PVC) was mixed with low concentrations of this copolymer and generated materials with silicone-like surface properties. *In vivo* studies showed that the PDMS-PCL-blended materials triggered significantly weaker inflammatory responses than did PVC				
Title:	RGD-coated titanium implants stimulate increased bone formation *in vivo*			**Author(s)/ Reference:**	Ferris DM et al., Biomaterials, 20(23-24), 1999, 2323-2331
Material:	Titanium rods coated with peptide sequence Arg-Gly-Asp-Cys	**Animal Model:**	Rats	**Implant Location:**	Femur

Summary:	This study was designed to evaluate the quality and quantity of new bone formed in response to titanium rods surface-coated with the peptide sequence Arg-Gly-Asp-Cys using gold-thiol chemistry and implanted in rat femurs. The pilot data suggested that an RGDC peptide coating may enhance titanium rod osseointegration				
Title:	*In vivo* evaluation of coralline hydroxyapatite and direct current electrical stimulation in lumbar spinal fusion			**Author(s)/ Reference:**	Bozic KJ et al., Spine, 24(20), 1999, 2127-2133
Material:	Coralline hydroxyapatite with an implantable dc electrical stimulator	**Animal Model:**	Rabbits	**Implant Location:**	Spine
Summary:	This study evaluated the effect of an osteoconductive bone graft substitute and direct-current electrical stimulation on the rate of pseudarthrosis in a rabbit spinal fusion model. Direct-current electrical stimulation increased fusion rates in a dose-dependant manner. Coralline hydroxyapatite and direct-current electrical stimulation can be used together to increase fusion rates.				
Title:	Experimental spinal fusion using sintered bovine bone coated with type I collagen and recombinant human bone morphogenetic protein-2			**Author(s)/ Reference:**	Minamide A et al., Spine, 24(18), 1999, 1863-1870
Material:	Sintered bovine bone and Type I collagen complex carrying recombinant human bone morphogenetic protein (rhBMP)-2	**Animal Model:**	Rabbits	**Implant Location:**	Lumbar
Summary:	This study examined the efficacy of sintered bovine bone coated with Type I collagen as a carrier of recombinant human bone morphogenetic protein-2 (RHBM P-2) for lumbar intertransverse process arthrodesis. Sintered bovine bone coated with Type I collagen and (RHBM P-2) resulted in a higher fusion rate than the autograft				
Title:	The use of coralline hydroxyapatite with bone marrow, autogenous bone graft, or osteoinductive bone protein extract for posterolateral lumbar spine fusion.			**Author(s)/ Reference:**	Boden SD, Spine, 24(4), 1999, 320-327.
Material:	Coralline hydroxyapatite with bone marrow, autogenous bone graft, osteoinductive bone protein extract	**Animal Model:**	Rabbits	**Implant Location:**	Spine
Summary:	This study examined the effectiveness of coralline hydroxyapitite as a bone graft substitute for lumbar spine fusion when used with bone marrow, autogenous bone graft, or an osteoconductove bone protein extract.				
Title:	Osseointegration of surface-blasted implants made of titanium alloy and cobalt-chromium alloy in a rabbit intramedullary model			**Author(s)/ Reference:**	Jinno T, et al., J. Biomedical Materials Research 1998, 42(1), 20-29.
Material:	Ti6Al4V and CoCr rods blasted with 710 μm Al2O3	**Animal Model:**	Rabbits	**Implant Location:**	Medullary space of distal femora
Summary:	The purpose of this stuffy was to compare the osseointegration of surface-blasted Ti6Al4V and CoCr implants *in vivo*. Ti6Al4V and CoCr rods blasted with 710 um Al2O3 particles were bilaterally press-fit into the medullary space of distal femora of rabbits. Both demonstrated good biocompatibility radiographically and histologically				
Title:	Ionomeric cement implants in the middle ear of the baboon (Papio ursinus) as a primate model			**Author(s)/ Reference:**	Geyer G, et al., European Archives of Oto-Rhino-Laryngology 1998, 255(8), 402-409.
Material:	Ionomeric cement	**Animal Model:**	Baboons	**Implant Location:**	Middle ear implants

Summary:	Implantation of hybrid bone substitute ionomeric cement in viscous or hardened physical states into the middle ears of a primate animal model was undertaken. Findings indicate that reconstruction of the posterior meatal wall can be used clinically				
Title:	The biological effect of natural bone mineral on bone neoformation on the rabbit skull			**Author(s)/ Reference:**	Hammerle CHF, et al., Clinical Oral Implants Research 1997, 8(3), 198-207.
Material:	Hemispherical dome made of polylactic acid filled either with peripheral blood or blood with OsteoGraf®	**Animal Model:**	Rabbits	**Implant Location:**	Calvaria
Summary:	The aim of this study was to evaluate the effect of deproteinized bovine bone graft material on new bone formation in a guided bone regeneration model system. The periosteal skin flap was raised in rabbits uncovering the calvaria. A stable hemispherical dome made of poly-lactic acid (PLA) was placed onto the roughened calvaria. It was concluded that deproteinized bovine bone mineral has osteoconductive properties and can initially accelerate new bone formation during guided bone regeneration by increased recruitment of osteoblasts				
Title:	Use of bone morphogenetic protein 2 on ectopic porous coated implants in the rat			**Author(s)/ Reference:**	Cole BJ, et al., Clinical Orthopaedics & Related Research 1997, 345, 219-228.
Material:	Cylindrical plasma sprayed porous titanium implants treated with recombinant human bone morphogenetic protein 2 with/without hydroxyapatite	**Animal Model:**	Rats	**Implant Location:**	Ectopic site - Quadriceps muscle pouch
Summary:	The ability of recombinant human bone morphogenetic protein-2 to remain osteoinductive and stimulate bone formation on a porous coated implant was tested in a rat quadricpes muscle pouch. Titanium implants were coated with hydroxyapitite. Conculsions suggested that recombinant human bone morphogenetic protein-2 remains biologically active after application to a titanium implant and may be used to enhance appositional bone formation by direct application to the implant surface				
Title:	Bovine-derived bone protein as a bone graft substitute in a canine segmental defect model			**Author(s)/ Reference:**	Sciadini et al., J. Orthopaedic Trauma, 11(7), 1997, 496-508.
Material:	Demineralised bone matrix with bovine-derived bone protein	**Animal Model:**	Dogs	**Implant Location:**	Radial defect
Summary:	The objective of this study was to evaluate the efficacy of a bone graft substitute in healing of a segmental defect of a weight-bearing long bone. Using an established canine model, a blinded, prospective, randomised study of the performance of bone graft substitute implants was compare with that of an accepted treatment modality (autograft) in a paired fashion. The conclusions of the study were that DBM+BP (demineralised bone matrix allograft plus bovine-derived bone protein) composite implants were more effecive at healing critical-sized segmental defects tan DBM alone				
Title:	Ceramic anterior spinal fusion. Biologic and biomechanical comparison in a canine model			**Author(s)/ Reference:**	Emery SE, Spine, 21(23), 1996, 2713-2719.
Material:	Hydroxyapatite ceramic, biphasic hydroxyapatite/tricalcium phosphate ceramic, calcium carbonate ceramic	**Animal Model:**	Dogs	**Implant Location:**	Spinal fusion

Summary:	This study compares the biomechanical stiffness and histologic appearance of fused spinal segments using ceramic graft substitutes versus autogenous bone grafts. The relative success or failure of ceramic grafts is influenced by the composition of the ceramic, location in the spine, stability and the animal model, which in this case is canine.				
Title:	Effects of different osteopromotive membrane porosities on experimental bone neogenesis in rats			**Author(s)/ Reference:**	Zellin G., et al., Biomaterials, 1996, 17(7), 695-702.
Material:	Dome-shaped expanded polytetrafluoroethylene membranes covered with periosteum and skin	**Animal Model:**	Rats	**Implant Location:**	Calvaria
Summary:	This study was performed to investigate the influence of polytetrafluorethylene (e-PTFE) membrane (GORE-TEX) porosity on the osteopromotive efficacy and to determine bone neogenesis in the rat. Three membrane qualities with different porosities and four healing periods were studied. Dome-shaped membranes were placed on denuded rat calvaria and covered with the periosteum and skin. Upon examination via histology, the results confirmed that it is possible to produce new bone by the use of the osteopromotion technique				
Title:	Preliminary experience with a novel model assessing *in vivo* mechanical strength of bone grafts and substitute materials			**Author(s)/ Reference:**	Hamson KR, Calcified Tissue International, 57(1), 1995, 64-68.
Material:	Allograft cortical bone, hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic granules and a HA/TCP and collagen composite	**Animal Model:**	Dogs	**Implant Location:**	Tibia
Summary:	A novel canine tibia model was used to evaluate four bone graft materials. Compared on the basis of peak torque, stiffness, and energy to peak torque, no significant differences were found among any of the graft materials or control bone. Histologic examination revealed the materials to be osteoconductive with the extensive formation of dense, compact, cancellous bone.				
Title:	Development of an elastomer coated hip prosthesis			**Author(s)/ Reference:**	Jaecques SV, J. Materials Sci – Materials in Medicine, 6(12), 1995, 685-689.
Material:	Elastomer coated prosthesis with a metal core and thermoplastic polyolefin elastomer coating	**Animal Model:**	Dogs	**Implant Location:**	Total hip replacement
Summary:	A cementless stem for a total hip replacement (THR) was designed aiming at some mechanical advantages of a cemented stem. It has a metal core coated with an elastomer layer (a thermoplastic polyolefin elastomer) as a mechanical buffer between the core and the femera I cortex. Stress distribution in photo-elastic models of the ensemble was studied and compared with a model of a cementless system. The core was tested for fatigue resistance in a simulator. Implantation of non-loaded samples in dogs has shown acceptable behaviour in contact with bone and marrow. The elastomer coating is being tested.				
Title:	The Effect Of Operative Fit And Hydroxyapatite Coating On The Mechanical And Biological Response To Porous Implants			**Author(s)/ Reference:**	Dalton JE, J. Bone Joint Surg. – Amer. Vol., 77A(1), 1995, 97-110.
Material:	Titanium-alloy (Ti-6Al-4V) porous bead coating onto a 2 mm diameter threaded rod with and without hydroxyapatite coating	**Animal Model:**	Dogs	**Implant Location:**	Femur

Summary:	Femoral intramedullary implants were constructed by threading disks with a titanium-alloy (Ti-6Al-4V) porous bead coating onto a 2 mm diameter threaded rod. Implants with and without a hydroxyapatite coating of 25 μm were inserted into 15 skeletally mature dogs. The femoral canal was sequentially reamed bilaterally to a 10 mm diameter, resulting in uniform initial implant-bone interface gaps of 0.0, 0.5, 1.0, and 2.0 mm. Three animals each were killed at 4, 8, 12, 24 and 52 weeks after the implantation. Characteristics of interface attachment were determined with test fixtures that supported the surrounding bone to within 150 μm of the interface. Histological sections were prepared, and the amount of bone within the porous structure and the amount of the original gap that was filled with new bone were quantified with a computerized video image-analysis system. The attachment strength and bone ingrowth are significantly affected by gaps in the interface, particularly those of more than 1.0 mm. Also, the use of a hydroxyapatite coating on the implants was shown to have a positive effect on fixation of the implant, especially when the initial gap was small.				
Title:	Hydroxylapatite/poly(L-lactide) composites: an animal study on push-out strengths and interface histology			**Author(s)/ Reference:**	Verheyen CCPM, J. Biomed. Mater. Res., 27(4), 1993, 433-444.
Material:	Hydroxylapatite and poly(L-lactide)	**Animal Model:**	Goats	**Implant Location:**	Transcortical implant
Summary:	Composites of hydroxylapatite and poly (L-lactide) (HA/PLLA) were tested in goats. Push-out testing of the implants indicated that PLLA reinforced with 50wt% hydroxylapatite or PLLA plasma-sprayed with a hydroxylapatite coating of 50um thickness increases interfacial shear strength at 3 months when compared to unfilled poly (L-lactide)				
Title:	Fibrillar collagen-biphasic calcium phosphate composite as a bone graft substitute for spinal fusion			**Author(s)/ Reference:**	Zerwekh JE, J. Orthopaed. Res., 10(4), 1992, 562-572.
Material:	Fibrillar collagen-biphasic calcium phosphate and autogenous bone	**Animal Model:**	Dogs	**Implant Location:**	Spine
Summary:	The osteoconductive capacity of fibrillar collagen-biphasic calcium phospate composition was compared to autogenous bone in a canine spinal fusion model. The quality of fusion in each animal assessed by biomechanical testing and histological analysis.				
Title:	A mechanical investigation of fluorapatite, magnesiumwhitlockite, and hydroxylapatite plasma-sprayed coatings in goats			**Author(s)/ Reference:**	Dhert WJA, J. Biomed. Mater. Res., 25(10), 1991, 1183-1200.
Material:	Ceramic coating of fluorapatite, magnesiumwhitlockite, hydroxylapatite on Ti-6Al-4V implants	**Animal Model:**	Goats	**Implant Location:**	Femur and humer
Summary:	Ceramic coating of fluorapatite (FA), magnesiumwhitlockite (MW), hydroxylapatite (HA), and non-coated Ti-6Al-4V alloy implants were evaluated before and after implantation in an animal study. Cylindrical plugs plasma-spray-coated with FA, MW and HA were implanted into the right femora and left humeri of goats. The *in vivo* results were evaluated using push-out tests and SEM. The FA and HA implants showed significantly higher push-out strengths than the MW and Ti alloy implants				
Title:	Hydroxyapatite-Ceramic For Juxtaarticular Implantation			**Author(s)/ Reference:**	Meenen NM, et al., J. Materials Science - Materials in Medicine 1992, 3(5), 345-351.
Material:	Hydroxyapatite-ceramic	**Animal Model:**	Rabbits	**Implant Location:**	Subchondral bone defects in medial femoral comdyles

Summary:	This study deals with the influence of a physiological load on the remodelling within hydroxyapatite (HA)-filled subchondral bone defects. The morphological aspect of functional adaptation of the hydroxyapatite-bone compound determined by the orientation of the bone collagen fibres were shown using polarising microscopy. By biomechanical methods, the elastic properties of the resulting ceramo-osseous regeneration complex were tested.

ANNEX 2 – *IN VIVO* STUDIES OF CARTILAGE MATERIALS

Title:	Foetal tracheal augmentation with cartilage engineered from bone marrow-derived mesenchymal progenitor cells			**Author(s)/ Reference:**	Fuchs JR, et al., J. Pediatric Surgery, 2003, 38(6), 984-987.
Material:	Ovine bone marrow-derived stromal cells seeded into polyglycolic acid polymer scaffolds	**Animal Model:**	Sheep	**Implant Location:**	Fetal trachea
Summary:	This study aimed to compare cartilage engineered from bone marrow-derived stromol cells				
Title:	Effect of lyophilized heterologous collagen on new cartilage formation from perichondrial flaps in rabbits: an experimental study			**Author(s)/ Reference:**	Ozbek S, et al., Annals of Plastic Surgery, 2003, 50(5), 528-534.
Material:	lyophilised heterologous collagen	**Animal Model:**	Rabbits	**Implant Location:**	Perichondrial flaps
Summary:	In this experimental study, *in vivo* effects of heterologous collagen sponge in perichondrial neochondrogenesis were examined in an animal model, and acceleration and enhancement effects were observed				
Title:	The use of absorbable co-polymer pads with alginate and cells for articular cartilage repair in rabbits			**Author(s)/ Reference:**	Cohen SB, et al., Biomaterials, 2003, 24(15), 2653-2660.
Material:	Rabbit rib chondrocytes in calcium alginate and co-polymer pads composed of polyglycolic acid and polylactic acid	**Animal Model:**	Rabbits	**Implant Location:**	Osteochondral defects in the knee
Summary:	This study evaluated the ability of polyglycolic acid (PGA)-polylactic acid (PLA) co-polymer pads with aliginate to deliver chondrocytes and influence osteochondral defect repair *in vivo* in rabbit knees. The conclusions of the study were that the addition of calcium alginate to the co-polymer pads offers a new approach to deliver cells to an osteochondral defect and may enhance cartilage regeneration				
Title:	*In vivo* mesenchymal cell recruitment by a scaffold loaded with transforming growth factor beta1 and the potential for in situ chondrogenesis			**Author(s)/ Reference:**	Huang Q, et al., Tissue Eng., 2002, 8(3), 469-482.
Material:	PCL scaffold and TGF-beta1-loaded fibrin glue	**Animal Model:**	Rabbits	**Implant Location:**	Ectopic sites for cartilage formation such as subcutaneous, intramuscular and subperiosteal
Summary:	The objectives of this study were (1) to develop a biphasic implant made of a bioresorbable polymeric scaffold in combination with TGF-beta1-loaded fibrin glue for tissue engineering applications, and (2) to determine whether the implant made of a polycaprolactone (PCL) scaffold and TGF-beta1-loaded fibrin glue could recruit mesenchymal cells and induce the process of cartilage formation when implanted in ectopic sites in rabbits. Conclusions were positive with respect to the above objectives				
Title:	Application of an X-ray microscopy technique to evaluate tissue-engineered bone-scaffold constructs			**Author(s)/ Reference:**	Schantz JT, et al., Mat. Sci. Eng. C-Biomimetic Supramolecular Systems, 2002, 20(1-2), 9-17.

Material:	PCL-HA construct with human calvarial corticicancellous bone cells	**Animal Model:**	Mice	**Implant Location:**	Ectopic site - back
Summary:	The aim of this study was to evaluate the feasibility of X-ray microscopy technique to analyze tissue-engineered samples. 3-D scaffolds were made of polycaprolactone (PCL)-hydroxylapatite (HA) (90/10 wt.%). A primary explant system of the morcellized grafts with cells from human calvarial corticocancellous bone was established within the PCL-HA constructs. Tissue constructs were cultured in vitro for 3 weeks and then implanted into the back of Balb C nude mice. Grafts were explanted after 17 weeks and tissue formation was assessed via XSAM and CT scan and histology.				
Title:	Thermogelling biodegradable copolymer aqueous solutions for injectable protein delivery and tissue engineering			**Author(s)/ Reference:**	Jeong B, Biomacromolecules, 2002, 3(4), 865-868.
Material:	Autogenic chrondrocyte cells suspended in PLGA-g-PEG aqueous polymer solution	**Animal Model:**	Rabbits	**Implant Location:**	Articular cartilage defects in right femoral trochlea
Summary:	This report shows that thermogelling biodegradable PLGA/PEG graft copolymer system can be a promising platform for protein and cell-based therapy using rats				
Title:	A tissue-engineering model for the manufacture of auricular-shaped cartilage implants			**Author(s)/ Reference:**	Haisch A, et al., European Archives of Oto-Rhino-Laryngology, 2002, 259(6), 316-321.
Material:	Silicone cylinder with human nasal septal chondrocytes crosslinked by human fibrin within bioresorbable PGLA-PLLA polymer scaffolds	**Animal Model:**	Mice	**Implant Location:**	Ectopic site - auricular reconstruction on backs of nude mice
Summary:	In this study, the reliability and quality of a tissue-engineering model for the manufacture of auricular-shaped human cartilage implants were investigated, focusing on the feasibility of the manufacturing process and the *in vivo* and in vitro maturation of an extracellular cartilage-like matrix. Implants were molded within and auricular-shaped silicone cylinder, and human nasal septal chondrocytes crosslinked by human fibrin within bioresorbable PGLA-PLLA polymer scaffolds were used				
Title:	Fluorescently labeled mesenchymal stem cells (MSCs) maintain multilineage potential and can be detected following implantation into articular cartilage defects			**Author(s)/ Reference:**	Quintavalla J, et al., Biomaterials, 2002, 23(1), 109-119.
Material:	Gelatin sponge seeded with flourescently labeled mesenchymal stem cells	**Animal Model:**	Goats	**Implant Location:**	Articular cartilage defect
Summary:	The short-term fate of fluorescently labelled mesenchymal stem cells (MSCs) after implantation into full-thickness cartilage defects *in vivo*. The labelling enabled researchers to determine whether the implanted cells were lost during early time points after implantation as well as their spatial orientation throughout the defect				
Title:	Articular cartilage repair using a tissue-engineered cartilage-like implant: an animal study			**Author(s)/ Reference:**	Mainil-Varlet P, et al., Osteoarthritis and Cartilage 2001, 9 S6-S15.
Material:	Cartilage-like implant (bioreactors (EU-Patent Nr. 922093, 1998) seeded with chondrocytes isolated from pigs)	**Animal Model:**	Minipigs	**Implant Location:**	Articular cartilage

Summary:	The aim of this study was to evaluate how well a tissue-engineered cartilage-like implant, derived from chondrocytes cultured in a novel patented, scaffold-free bioreactor system, would perform in minipig knees with chondral, superficial, osteochondral, and full-thickness articular defects. The conclusion of the study was that repair of cartilage defects with this implant yielded a consistent gross cartilage repair with a matrix predominantly composed of type II collagen up to 6 months after implantation				
Title:	A biomechanical analysis of an engineered cell-scaffold implant for cartilage repair			**Author(s)/ Reference:**	Peretti GM, et al., Annals of Plastic surgery, 2001, 46(5), 533-537.
Material:	Lyophilised articular cartilage chips mixed with a cell-fibrinogen solution and thrombin to obtain constructs made of fibrin glue	**Animal Model:**	Nude mice	**Implant Location:**	Ectopic site - subcutaneous tissue
Summary:	This study evaluated the biomechanical and physical properties of newly formed cartilage from isolated chondrocytes in combination with matrix components. The study demonstrated that adding lyophilised cartilage chips to a fibrin glue-engineered cartilage construct maintains the biomechanical properties and the original mass after medium-/long-term *in vivo* transplantation				
Title:	A new *in vivo* model for testing cartilage grafts and biomaterials: the 'rabbit pinna punch-hole' model			**Author(s)/ Reference:**	ten Koppel PGJ, et al., Biomaterials, 2001, 22(11), 1407-1414.
Material:	Demineralised bovine bone matrix (DBM), DBM in perichondrium envelope and DBM seeded with isolated chondrocytes	**Animal Model:**	Rabbits	**Implant Location:**	Punch-holes in the external ears
Summary:	An animal model was developed for evaluation of the feasibility of cartilage grafts. The rabbit pinna punch-hole model was seen to be a reliable and efficient method for first evaluation of cartilage grafts				
Title:	Osteochondral reconstruction of a non-weight-bearing joint using a high-density porous polyethylene implant			**Author(s)/ Reference:**	Weinzweig J, et al., Plastic and Reconstructive Surgery, 2000, 106(7), 1547-1554.
Material:	Medpor (high-density porous polyethylene)	**Animal Model:**	Chickens	**Implant Location:**	Osteochondral defect
Summary:	MedPor is a high-density porous polyethylene (HDPP) scaffold biomaterial. An avian non-weight-bearing joint model was designed to study the role of the HDPP implant in small joint reconstruction. Joints in the HDPP implant group demonstrated stable fixation by highly mineralised bony trabecular ingrowth, preservation of th articular contour of the humeral head, and no evidence of significant degenerative joint disease				
Title:	Internal support of tissue-engineered cartilage			**Author(s)/ Reference:**	Arevalo-Silva CA, et al., Archives of Otolaryngology - Head & Neck Surgery, 2000, 126(12), 1448-1452.
Material:	High-density polyethylene, soft acrylic, polymethylmethaclylate, extra purified Silastic and Conventional Silastic seeded with chondrocytes isolated from the pigs and enveloped by Pluronic F-127	**Animal Model:**	Pigs	**Implant Location:**	Subdermal implants

Summary:	This report tested the hypothesis that tissue-engineered autologous cartilage can be bioincoporated with a nonreactive, permament endoskeletal scaffold. Auricular elastic cartilage was harvested from swine. The chondrocytes were isolated and suspended into a hydrogel. Nonbiodegradable endoskeletal scaffolds were formed by the materials above. This pilot technique demonstrated success in limiting the inflammatory response to the scaffold, especiialy to high-density polyethylene, acrylic, and extrapurified Silastic				
Title:	Engineering autogenous cartilage in the shape of a helix using an injectable hydrogel scaffold			**Author(s)/ Reference:**	Saim AB, et al., Laryngoscope 2000, 110(10), 1694-1697.
Material:	Autogenous chondrocytes suspended in of Pluronic F-127 (polyethylene oxide and polypropylene oxide) hydrogel. Contour of the implant supported by a skin fold channel in the shape of the helix of a human ear created in the skin. Cell-hydrogel suspension was injected through the skin fold channel	**Animal Model:**	Pigs	**Implant Location:**	Ventral surface - autogenous cartilage in the shape of a human ear helix using injectable hydrogel scaffolding
Summary:	Autogenous cartilage in the shape of a human ear has been engineered. This preliminary method extends the concept of auricular tissue engineering from an immunocompromised xeongraft model to an immunocompetent autologous animal model				
Title:	Cartilage tissue engineering for ear as in rabbit model with perforated polyurethane prosthesis: In vivo assay			**Author(s)/ Reference:**	Mirzadeh H, et al., Iranian Polymer Journal, 2000, 9(2), 73-80.
Material:	Perforated polyurethane prosthesis	**Animal Model:**	Rabbits	**Implant Location:**	External ear
Summary:	Attempts have been done in this study to assay in vivo implantation of the perforated polyurethane prosthesis as ear cartilage in a rabbit model. After 1 to 5.5 months en bloc resection of prosthesis and surrounding tissues for histopathological and biocompatibility studies were performed. Of the 18 inserted implants, complete healing was observed in 8 (45%) cases. Four (22%) of the remaining 10 implants showed partial healing and remaining six (33%) showed non-healing. It was, therefore, concluded that perforation might improve the cartilage cell ingrowth.				
Title:	Degree of differentiation of chondrocytes and their pretreatment with platelet-derived-growth factor. Regulating induction of cartilage formation in resorbable tissue carriers *in vivo*			**Author(s)/ Reference:**	Lohmann CH, et al., Orthopade 2000, 29(2), 120-128.
Material:	Scaffolds with 80% poly(D,L-lactide-co-glycolide (PLG)) with a 75:25 lactide:glycolide ratio and 20% modified PLG with a 50:50 lactide:glycolide ratio seeded with cells derived from either the femoral articular cartilage, costochondral periochondrium or costochondral rest zone cartilage of rats	**Animal Model:**	Nude mice	**Implant Location:**	Ectopic site- calf muscle
Summary:	In this study, the ability of cells from articular cartilage, perichondrium, and costochondral resting zone to form new cartilage when loaded onto biodegradable scaffolds and implanted into calf muscles of mice. The results showed that resting zone cells can be successfully incorporated into biodegradable porous poly(D,L-lactide-co-glycolide) (PLG) scaffolds and can induce new cartilage formation in a nonweight-bearing site.				

Title:	Articular cartilage repair in rabbits by using suspensions of allogenic chondrocytes in alginate			**Author(s)/ Reference:**	Fragonas E, et al., Biomaterials 2000, 21(8), 795-801.
Material:	Alginate gels with allogenic chondrocytes, which was gelled in situ by the addition of calcium chloride solution directly into defects	**Animal Model:**	Rabbits	**Implant Location:**	femoral condyle
Summary:	The feasibility of allogenic implants of chondrocytes in alginate gels was tested for the resconstruction *in vivo* of artificially full-thickness-damaged articular rabbit cartilage. A complete repair of the defect was observed 4-6 months from the implant of the chondrocytes with the recovery of a normal tissued structure				
Title:	Pretreatment with platelet derived growth factor-BB modulates the ability of costochondral resting zone chondrocytes incorporated into PLA/PGA scaffolds to form new cartilage *in vivo*			**Author(s)/ Reference:**	Lohmann CH, et al., Biomaterials 2000, 21(1), 49-61.
Material:	Scaffolds constructed from copolymers of polylactic acid and polyglycolic acid (either made more hydrophilic (foam) or re-enforced with 10% polyglycolic acid fibres to increase stiffness).	**Animal Model:**	Nude Mice	**Implant Location:**	Ectopic site- calf muscle
Summary:	This study examined the ability of chondrocytes to support cartilage formation when incorporated into biodegradable scaffolds constructed from copolymers (PLG) of polylactic acid (PLA) and polyglycolic acid (PGA) and implanted in the calf muscle of mice. The results indicate that in this model, costochondral resting zone chondrocytes (RC) produce cartilage; pre-treatment of the RC cells with recombinant human platelet derived growth factor-chondrogenic cells (PDGF-BB) promotes retention of a lyaline-lie chondrocyte phenotype; and the material properties of the implant do not negatively impact on the ability of the cells to support chondrogenesis				
Title:	Experimental lead arthropathy: an animal model			**Author(s)/ Reference:**	Harding NR, et al., J. Trauma-Injury Infection and Critical Care 1999, 47(5), 951-955.
Material:	Lead or stainless steel pellets	**Animal Model:**	Rabbits	**Implant Location:**	Intraarticular knee joints
Summary:	This study examined the long-term effects of intra-articular lead on joint structures in an animal model. Rabbits had identical lead or stainless steel pellets inserted into both knees. Intrasynovial lead has been linked to lead intoxification. Thus study supports removal of lead bodies from articular areas in an attempt to reduces or slow the degeneration of the affected joints. Nonmechanical effects of lead on inraarticular structures may lead to dengenerative changes				
Title:	Autologous engineered cartilage rods for penile reconstruction			**Author(s)/ Reference:**	Yoo JJ, et al., J. Urology 1999, 162(3), 1119-1121.
Material:	Poly-L-lactic acid coated polyglycolic acid rods seeded with autologous chondrocytes from rabbit ears	**Animal Model:**	Rabbits	**Implant Location:**	Corpus cavernosum
Summary:	Previous studies have shown that rods composed of cartilage could be created using chondrocytes seeded on biodegradable polymer scaffolds. These rods are readily elastic and withstand high degrees of pressure. The feasibilyu of applying the engineered rods in situ in an animal model is investigated here. Autologous chondrocytes seeded on these preformed biodegradable polymer structures form cartilage structures within the rabbit corpus cavernosum.				

Title:	Hyaluronic acid-based polymers as cell carriers for tissue-engineered repair of bone and cartilage			**Author(s)/ Reference:**	Solchaga LA, et al., J. Orthopaedic Research 1999, 17(2), 205-213.
Material:	HYAFF 11 and ACP sponges (based on hyaluronic acid modified by esterification of the carboxyl groups of the glucuronic acid) seeded with bone marrow-derived mesenchymal progenitor cells	**Animal Model:**	Nude mice	**Implant Location:**	Ectopic- Subcutaneous
Summary:	HYAFF 11 and ACP sponges, two biomaterials based on hyaluronic acid modified by esterification of the carboxyl groups of the glucuronic acid, were tested as osteogenic or chondrogenic delivery vehicles for rabbit mesenchymal progenitor cells and compared with a well characterised porous calcium phosphate ceramic delivery vehicle. The results showed that the sponges allowed incorporated twice as many cells and produced a 30% increase in the relative amount of bone and cartilage per unit area.				
Title:	The role of trabecular demineralized bone in combination with perichondrium in the generation of cartilage grafts			**Author(s)/ Reference:**	van Osch GJVM, et al., Biomaterials 1999, 20(3), 233-240.
Material:	Bovine trabecular demineralised bone matrix and perichondrium	**Animal Model:**	Rabbits	**Implant Location:**	External ear
Summary:	The use of a composite graft of bovine trabecular demineralised bone matrix (DBM) and perichomdrium has been found to be a reliable method for *in vivo* generation of cartilage. In this study, the mechanism whereby this commercially available matrix increases cartilage formulation was investigated.				
Title:	Tissue-engineered nipple reconstruction			**Author(s)/ Reference:**	Cao YL, et al., Plastic and Reconstructive Surgery 1998, 102(7), 2293-2298.
Material:	Co polymer of polyethylene oxide and polypropylene (Pluronic F-127) with autologous chondrocytes	**Animal Model:**	Pigs	**Implant Location:**	Cartilage in the shape of a human nipple
Summary:	An approach to the creation of autologous tissue-engineered cartilage in the shape of a human nipple by injecting a reverse thermosensitive polymer seeded with autologous chondrocytes in an immumocompetent porcine animal model is described.				
Title:	Development of a novel osteochondral graft for cartilage repair			**Author(s)/ Reference:**	Toolan BC, et al., J. Biomedical Materials Research 1998, 41(2), 244-250.
Material:	Bovine osteochondral graft treated with enzymatic digestion and solvent-treatment was used as a carrier for bovine chondrocytes	**Animal Model:**	Rabbits	**Implant Location:**	Articular cartilage in knee joint
Summary:	This study reports the development of a novel osteochondral graft for cartilage repair. The xenograft provoked a mild inflammatory response; however, this did not impede the repair process				
Title:	Autogenous tissue-engineered cartilage: evaluation as an implant material			**Author(s)/ Reference:**	Britt JC and Park SS, Archives of Otolaryngology - Head & Neck Surgery 1998, 124(6), 671-677.

Material:	Autogenous chondrocytes seeded onto polyglycolic acid-poly-L-lactic acid copolymer templates	**Animal Model:**	Rabbits	**Implant Location:**	Sculpted cartilage in the shape of cross, nasal tip graft or auricle
Summary:	The objectives of this study were to determine whether autogenous tissue-engineered cartilage grafts can be synthesised in predetermined shapes, to compare tissue-engineered cartilage with native cartilage with respect to histological characteristics and biomechanical properties, and to demonstrate how multiple transplantations affect tissue-engineered cartilage. Autogenous chondrocytes were seeded onto biodegradable polyglycolic acid-poly-L-lactic acid copolymer templates in 1 of 3 shapes. Grafts and controls of sculpted cartilage were divided amongst three groups of rabbits. Production of tissue-engineered cartilage was confirmed in 30 of 31 implants				
Title:	Transplantation of allograft chondrocytes embedded in agarose gel into cartilage defects of rabbits			**Author(s)/ Reference:**	Rahfoth B, et al., Osteoarthritis and Cartilage 1998, 6(1), 50-65.
Material:	Allografts of cultured chondrocytes in agarose gels	**Animal Model:**	Rabbits	**Implant Location:**	Articular cartilage defects in knees
Summary:	In this study, a new approach to repairing articular cartilage defects in rabbit knees by allografting chondrocytes cultured in agarose gels is described. The study demonstrated that agarose-embedded chondrocyte may prove a valuable tool for controlled repair of articular cartilage defects				
Title:	Porous high-density polyethylene implants in auricular reconstruction			**Author(s)/ Reference:**	Williams JD, et al., Archives of Otolaryngology - Head & Neck Surgery 1997, 123(6), 578-583
Material:	Porous high-density polyethylene (Medpor) implants covered with a skin flap	**Animal Model:**	Rabbits	**Implant Location:**	Articular cartilage defect
Summary:	Polyethylene implants (Medpor) were placed in surgically created defects in auricular cartilage in rabbits, covered with a skin flap and then exposed at various times after implantation. The study concluded that polyethylene implants are well tolerated as replacements for native cartilage in auricular construction				
Title:	Foreign-body reaction and the course of osteolysis after polyglycolide implants for fracture fixation: experimental study in sheep			**Author(s)/ Reference:**	Weiler A, et al., J. Bone & Joint Surgery - British Vol. 1996, 78B(3), 369-376.
Material:	Self-reinforced polyglycolide rods (Biofix)	**Animal Model:**	Sheep	**Implant Location:**	Osteochondral fracture of the medial femoral condyle
Summary:	This study examined the effects of polyglycolide (PGA) rods in 12 sheep with standardised osteochondral fractures of the medial femoral condyle fixed with uncoloured, self-reinforced PGA rods (Biofix). The fractures healed reliably; however, there were frequent, significant foreign-body reactions				
Title:	Articular cartilage repair using allogeneic perichondrocyte-seeded biodegradable porous polylactic acid (PLA): a tissue-engineering study			**Author(s)/ Reference:**	Chu CR, et al., J. Biomedical Materials Research 1995, 29(9), 1147-1154.
Material:	Porous D,D-L,L-polylactic acid seeded with allogeneic perichondrocyte	**Animal Model:**	Rabbits	**Implant Location:**	Osteochondral defects in medial femoral condyles

Summary:	The purpose of this study is to determine the suitability of porous D,D-L,L-polylactic acid as a carrier for delivering repair cells obtained from rib perichondrium into full-thickness articular cartilage defects. Results suggest that these scaffolds support the growth of cartilaginous repair tissue and are compatible with both in vitro and *in vivo* survival of chondrogenic cells				
Title:	*In vivo* chondrogenesis in collagen sponge sandwiched by perichondrium			**Author(s)/ Reference:**	Matsuda K, et al., J. Biomaterials Sc. - Polymer Edition 1995, 7(3), 221-229.
Material:	Auricular perichondrium with a collagen sponge	**Animal Model:**	Rabbits	**Implant Location:**	Graft on back
Summary:	In order to increase the cartilage synthesis of the perichondrium, this study combined auricular perichondrium with a collage sponge as a template and implanted the assembly into the back of rabbits. Microscopic examination revealed that cartilaginous tissue was produced in the collagen sponge and chondrosyntheis was accelerated in the collagen sponge implants in comparison with that in materials containing the perichondrium alone				
Title:	Temporomandibular joint disc replacement made by tissue-engineered growth of cartilage			**Author(s)/ Reference:**	Puelacher WC, et al., J. Oral & Maxillofascial Surgery 1994, 52(11), 1172-1177.
Material:	Polylactide and polyglycolic acid fibres used in the shape of temporomandibular joint seeded with bovine articular cartilage-derived chondrocytes	**Animal Model:**	Nude mice	**Implant Location:**	Subcutaneous
Summary:	To test the effectiveness of the technique of tissue-engineered growth of cartilage, termpromandibular joint (TMJ) disc replacements were created by seeding dissociated chondrocytes on synthetic, three-dimensional, bioresorbable polymer constructs. These cell-polymer constructs were implanted subcutaneously into mice. All implants seeded with chondrocytes showed gross evidence of histologically organised hyaline cartilage. The scaffolds maintained their specific shape				
Title:	Joint resurfacing using allograft chondrocytes and synthetic biodegradable polymer scaffolds			**Author(s)/ Reference:**	Freed LE, et al., J. Biomedical Materials Research 1994, 28(8), 891-899.
Material:	Fibrous polyglycolic acid scaffolds seeded with allogenic articular chondrocytes	**Animal Model:**	Rabbits	**Implant Location:**	Defects in knee joints
Summary:	Cartilage implant which could potentially be used to resurface damaged joints were created using rabbit articular chondrocytes and synthetic, biodegradable polymer scaffolds. Cells were serially passaged and then cultured in vitro on fibrous polyglycolic acid (PGA) scaffolds. Cell-PGA constructs were implanted *in vivo* in the knee joints of rabbits. This pilot study demonstrated that is is feasible to use cell-polymer allografts for joint resurfacing *in vivo*				
Title:	A Novel In-Vivo Model For The Study Of Cartilage Degradation			**Author(s)/ Reference:**	Bishop J, et al., J. Pharmacological and Toxicological Methods 1993, 30(1), 19-25.
Material:	Cylinder of bovine nasal cartilage positioned in the centre of a sponge which was pretreated with an irritant	**Animal Model:**	Rats	**Implant Location:**	Subcutaneous

Summary:	A cylinder of bovine nasal cartilage was positioned in the center of a sponge which had been pretreated with an irritant. These were then implanted subcutaneously into the backs of rats for periods of up to 16 days. The implanted sponges were rapidly surrounded by granulation tissue, maximal on day 2, and infiltrated by inflammatory cells which reached peak levels on day 9. Analysis of the cartilage shows an initial increase in wet weight and rapid loss of glycosaminoglycans. These changes were later followed by loss of cartilage wet weight and significant loss of hydroxyproline content. In a separate study, the effects of Mycobacterium tuberculosis (Mtb), kaolin, and zymosan were compared (1 mg/sponge) and the results showed that only Mtb induced pronounced inflammation and degradation of cartilage. The cartilage degradation directly correlated with the granulation tis sue weight, but not with cellular infiltration.				
Title:	Meniscal Regeneration With Copolymeric Collagen Scaffolds – In vitro And In vivo Studies Evaluated Clinically, Histologically, And Biochemically			**Author(s)/ Reference:**	Stone KR, et al., American Joumal of Sports Medicine 1992, 20(2), 104-111.
Material:	Copolymeric collagen-based scaffold	**Animal Model:**	Immature pigs and mature dogs	**Implant Location:**	Knee joint meniscus
Summary:	A resorbable collagen-based scaffold was designed and used in vitro and in vivo studies. In vivo, the scaffold was implanted in the knees of immature swine and mature canines and evaluated clinically, histologically, and biochemically. All dogs underwent an 80% subtotal resection of the medial meniscus bilaterally. A collagen template was implanted in one stifle (N = 24). The results demonstrated that a copolymeric collagen-based scaffold is compatible with meniscal fibrochondrocyte growth in vitro and in vivo, that does not inhibit meniscal regeneration in an immature pig, and that may induce regeneration of the meniscus in the mature dog.				
Title:	The role of polyglycolic acid rods in the regeneration of cartilage from perichondrium in rabbits			**Author(s)/ Reference:**	Ruuskanen MM, et al., Scandinavian J. Plastic & Reconstructive Surgery & Hand Surgery 1991, 25(1), 15-18.
Material:	Self-reinforced polyglycolic acid rod replaced the resected cartilage and retained perichindrium was sutured around the implant	**Animal Model:**	Rabbits	**Implant Location:**	Fifth rib cartilage was resected subperichondrially
Summary:	The role of polyglycolic acid (PGA) rods in the regeneration of cartilage from perichondrium was investigated in rabbits. Pronounced neocartilage formation was seen and grew up to form a tube around the implant				

ANNEX 3 – *IN VIVO* STUDIES OF INTERVERTEBRAL DISC MATERIALS

Title:	Titanium or polymethylmethacrylate in cervical disc surgery? A prospective study.			**Author(s)/ Reference:**	Jollenbeck B, Fernandez N, Firsching R. Zentralbl Neurochir. 2001; 62(4): 200-2.
Material:	Disc manufactured from polymethylmethacrylate. (Interbody fusion using Titanium cage)	**Animal Model:**	Humans	**Implant Location:**	Total disc replacement
Summary:	In 200 patients with either isolated protrusion of disc or with spondylosis a cervical discectomy in one or two levels was performed from a ventral approach. In 100 patients the removed disc was replaced with an implant of polymethylmethacrylat (PMMA). In 100 patients interbodyfusion was done with a titanium cage. Both groups were analysed in a prospective study. Clinical outcome was assessed after surgery. Results were similar in terms of complications and clinical outcome. As the PMMA surgery took longer and the costs of titanium are higher, there is no marked advantage of one implant material over the other.				
Title:	Use of bioceramics in the treatment of fractures of the thoraco-lumbar spine			**Author(s)/ Reference:**	Stulik J, Krbec M, Vyskocil T., Acta Chir Orthop Traumatol Cech. 2002; 69(5): 288-94.
Material:	Investigation using bioceramic granules to provide material for replacing osseous tissue in the body of vertebra as well as providing the conditions necessary for bone restructuring.	**Animal Model:**	Humans	**Implant Location:**	Use of bioceramic granules as osteoconductive materials in repairing damaged spines
Summary:	The aim of the study was to assess the effect of BAS-O bioceramic granules, inserted by transpedicular approach, on the development of post-operative kyphosis of the segments injured. The conclusions of the study were that bioceramic granules provide material for replacement of osseous tissue in the body of the vertebra as well as conditions necessary for bone restructuring. The loss of correction per segment was lower by about half in patients treated with bioceramic granules than in those who received a spongeous bone grafts.				
Title:	A composite bone graft substitute for anterior cervical fusion: assessment of osseointegration by quantitative computed tomography			**Author(s)/ Reference:**	Papavero L, Zwonitzer R, Burkard I, Klose K, Herrmann HD. Spine. 2002 May 15; 27(10): 1037-43.
Material:	Rectangular fenestrated titanium cage filled with a highly porous hydroxyapatite cylinder soaked with bone marrow	**Animal Model:**	Humans	**Implant Location:**	Anterior cervical fusion with a bone graft substitute
Summary:	The objective of this study was to investigate the clinical efficacy and osseointegration of an anterior cervical fusion with a bone graft substitute. The composite bone graft substitute consisted of a rectangular fenestrated titanium cage filled with a highly porous hydroxyapatite cylinder soaked with bone marrow aspirated from a vertebra. Altogether, 102 implants were inserted for anterior cervical fusion. The bone ingrowth was measured in 50 patients by quantitative computed tomography of the implant and the adjacent vertebrae after 1 week, then 6 and 12 months after surgery. The investigation was repeated in 24 randomly selected patients 24 months after surgery.				

Title:	Bone ingrowth fixation of artificial intervertebral disc consisting of bioceramic-coated three-dimensional fabric			**Author(s)/ Reference:**	Takahata M, Kotani Y, Abumi K, Shikinami Y, Kadosawa T, Kaneda K, Minami A. Spine. 2003 Apr 1; 28(7): 637-44; discussion 644
Material:	Bone-bonding characteristics of an intervertebral disc manufactured from bioceramic-coated three-dimensional fabric	**Animal Model:**	Sheep	**Implant Location:**	Artificial intervertebral disc
Summary:	The bone-bonding characteristic of a new artificial intervertebral disc consisting of bioceramic-coated three-dimensional fabric was evaluated mechanically and histologically in an in vivo sheep model.				
Title:	Replacement of the vertebral body with an expansion implant (Synex)			**Author(s)/ Reference:**	Krbec M, Stulik J, Tichy V. Acta Chir Orthop Traumatol Cech. 2002; 69(3): 158-62.
Material:	Synex (an in situ expandable vertebral body replacement implant. made of titanium alloy)	**Animal Model:**	Humans	**Implant Location:**	Replacement of the vertebral body
Summary:	This paper describes replacement of the vertebral body with the expansion implant Synex. In 25 cases, the vertebral body replacement was completed by posterior stabilization using internal fixation and, in nine cases, by anterior stabilization with a Ventrofix fixator. In 32 patients, the implant was inserted from the anterior approach and, in two, from the posterior approach following complete spondylectomy.				
Title:	Use of bioactive glass ceramics in the treatment of tibial plateau fractures			**Author(s)/ Reference:**	Urban K., Acta Chir Orthop Traumatol Cech. 2002; 69(5): 295-301.
Material:	Glass-ceramics BAS-O used in combination with autogenous bone marrow	**Animal Model:**	Humans	**Implant Location:**	Surgical treatment of proximal tibia fractures
Summary:	The surgical treatment of proximal tibia fractures requires a reduction and fixation of the split condyle, elevation of the depressed interfragment of articular surface. The golden standard is the application of autogenous bone grafts for filling in the defect in the cancellous bone of the tibial condyle. Bioactive glass-ceramics material may be successfully used for the filling. Gass-ceramics BAS-O is biocompatible and bioactive and has also suitable mechanical properties and therefore in this study it was used in combination with autogenous bone marrow in order to speed up osteogenesis.				
Title:	Anterior lumbar intervertebral fusion with artificial bone in place of autologous bone.			**Author(s)/ Reference:**	Xu W, Chen A, Feng X, Yin W. J Huazhong Univ Sci Technolog Med Sci. 2003; 23(3): 300-1
Material:	Porous hydroxyapatite (HA)/ZrO2 ceramics loading bone morphogenetic protein (BMP)	**Animal Model:**	Rabbits	**Implant Location:**	Feasibility assessment of anterior lumbar intervertebral fusion with artificial bone in place of autogenous bone

Summary:	The feasibility of anterior lumbar intervertebral fusion with artificial bone in place of autogenous bone was investigated. Porous hydroxyapatite (HA)/ZrO2 ceramics loading bone morphogenetic protein (BMP) were implanted after removal of lumbar vertebral disc in rabbits. The adjacent intervertebral discs were also removed by the same way and autogenous illic bone was implanted. SEM observation and biomechanical test were carried out. Compound bone had a bit lower osteoinductive activity than autogenous bone by SEM (Osteoinductive activity of artificial bone in 12 weeks was the same as that of autogenous bone in 9 weeks). Biomechanical test revealed that compound bone had lower anti-pull strength than autogenous bone ($P < 0.001$), but there was no significant difference in anti-pull strength between compound bone at 12th week and autogenous bone at 9th week ($P > 0.05$). It was concluded that compound bone could be applied for anterior spinal fusion, especially for those patients who can't use autogenous bone.				
Title:	Results of lumbar disk prosthesis after a follow-up period of 48 months.			**Author(s)/ Reference:**	Caspi I, Levinkopf M, Nerubay J. Isr Med Assoc J. 2003 Jan; 5(1): 9-11
Material:	Charite SB III disk prosthesis	**Animal Model:**	Humans	**Implant Location:**	Lumbar disk replacement with the Charite SB III disk prosthesis
Summary:	The objectives of the study were to report the outcome of the artificial lumbar disk replacement with the Charite SB III disk prosthesis in 20 patients after a 48 month follow-up. Eighty percent of patients reported satisfactory to very good results.				
Title:	An atelocollagen honeycomb-shaped scaffold with a membrane seal (ACHMS-scaffold) for the culture of annulus fibrosus cells from an intervertebral disc.			**Author(s)/ Reference:**	Sato M, Asazuma T, Ishihara M, Kikuchi T, Masuoka K, Ichimura S, Kikuchi M, Kurita A, Fujikawa K. J Biomed Mater Res. 2003 Feb 1; 64A(2): 248-56.
Material:	Atelocollagen gel	**Animal Model:**	Rabbits	**Implant Location:**	Use of atelocollagen honeycomb-shaped scaffold with a membrane seal for culture of annulus fibrosus cells in tissue engineering procedures of intervertebral disc repair
Summary:	The aim of this study was to investigate the possibility of using the atelocollagen honeycomb-shaped scaffold with a membrane seal (ACHMS-scaffold) for the culture of annulus fibrosus (AF) cells in tissue engineering procedures of intervertebral disc repair. cells from the intervertebral discs of Japanese white rabbits were cultured for up to 3 weeks in the ACHMS-scaffold to allow a high density, three-dimensional culture. The AF cells are able to grow and remain phenotypically stable in the scaffold.				
Title:	History, design and biomechanics of the LINK SB Charite artificial disc.			**Author(s)/ Reference:**	Link HD. Eur Spine J. 2002 Oct; 11 Suppl 2: S98-S105. Epub 2002 Sep 05.
Material:	Charite disc - a double coating of titanium/calciumphosphate UHMWPE (Ultra High Molecular Weight Polyethylene) sliding core	**Animal Model:**	Humans	**Implant Location:**	Disc repair

Summary:	The SB Charite I artificial disc was developed in 1982 by Schellnack and Buttner-Janz and modified as the Mark II version in 1984. Both types were manufactured in the former German Democratic Republic (GDR). Today's design, the SB Charite III, was first produced by LINK in 1987. Five sizes of the artificial disc in various angulations are available today, with a double coating of titanium/calciumphosphate. Designed with a three-component set-up, the SB Charite mimics the physiological segmental motion. The possibility of translation in the SB Charite provides proper biomechanical function and protects the zygapophysial joints. Results of biomechanical testing showed a sufficient cold-flow resistance of the UHMWPE (Ultra High Molecular Weight Polyethylene) sliding core and confirmed the negligible abrasion rate. The LINK SB Charite disc is a safe and effective operative treatment for discogenic low back pain. Long-term results (10 years and more) have been published.				
Title:	Artificial nucleus replacement: clinical experience			**Author(s)/ Reference:**	Klara PM, Ray CD. Spine. 2002 Jun 15; 27(12): 1374-7.
Material:	Device consists of a hydrogel core encased in a polyethylene jacket	**Animal Model:**	Humans	**Implant Location:**	Prosthetic disc nucleus
Summary:	The prosthetic disc nucleus is designed to help treat patients suffering from degenerative disc disease. The device consists of a hydrogel core that is encased in a polyethylene jacket and is intended to restore disc height while permitting normal range of motion. Clinical trials for the prosthetic disc nucleus were first conducted in 1996, and the device was found to be effective in most of the patients that were implanted. Subsequent changes have been made to device shapes and to the surgical protocol to facilitate implantation, thereby eliminating the high device migration rates.				
Title:	Artificial intervertebral disc replacement using bioactive three-dimensional fabric: design, development, and preliminary animal study.			**Author(s)/ Reference:**	Kotani Y *et al* Spine. 2002 May 1; 27(9): 929-35; discussion 935-6.
Material:	Triaxial three-dimensional fabric (3-DF) woven with an ultra-high molecular weight polyethylene fiber, and spray-coated bioactive ceramics on the disc surface	**Animal Model:**	Sheep	**Implant Location:**	artificial intervertebral disc
Summary:	A new artificial intervertebral disc was developed, and its intrinsic biomechanical properties, bioactivity, and the effectiveness as a total disc replacement were evaluated in vitro and *in vivo*. The artificial intervertebral disc consists of a triaxial three-dimensional fabric (3-DF) woven with an ultra-high molecular weight polyethylene fiber, and spray-coated bioactive ceramics on the disc surface. Conclusions were that an artificial intervertebral disc using a three-dimensional fabric demonstrated excellent in vitro and *in vivo* performance in both biomechanics and interface histology. There is a potential for future clinical application.				
Title:	3-dimensional biomechanical study of a new flexible lumbar intervertebral disk implant			**Author(s)/ Reference:**	Zollner J, Heine J, Eysel P. Orthopade. 2001 May; 30(5): 323-7.
Material:	Discs (polymethyl siloxane polymer, DR-PMSO)	**Animal Model:**	Calf cadaver	**Implant Location:**	artificial nucleus implant for lumbar discs
Summary:	The aim of this study was to determine the significance of a new artificial nucleus implant for lumbar discs (polymethyl siloxane polymer, DR-PMSO) with special regard to the biomechanical properties of the spinal motion segment.				
Title:	Artificial disc replacement. Preliminary report with a 3-year minimum follow-up.			**Author(s)/ Reference:**	Enker P, Steffee A, Mcmillin C, Keppler L, Biscup R, Miller S. Spine. 1993 Jun 15; 18(8): 1061-70.

Material:	The Acroflex disc (Acromed Corporation, Cleveland, OH (rubber core vulcanized to two titanium end plates that have superior posts to provide initial mechanical fixation and porous in-growth surfaces for long-term fixation	**Animal Model:**	Humans	**Implant Location:**	Artificial disc replacement
Summary:	Artificial disc replacement was performed in six patients with an average age of 55 years and average follow-up of 3.4 years. Four of the six patients had juxtafusion degeneration, one had multilevel disc degeneration, and one patient had isolated disc resorption. The Acroflex disc (Acromed Corporation, Cleveland, OH), which was used in the replacement, is composed of a rubber core vulcanized to two titanium end plates. The latter have superior posts to provide for initial mechanical fixation and porous in-growth surfaces for long-term fixation. Satisfactory results occurred in four of six patients. Poor results occurred in the presence of deformity that resulted in prosthetic failure and isolated disc resorption. There was an average 8 degrees angular and 2.3-mm translational movement and satisfactory in-growth at all interfaces. Design objectives of endurance, biocompatibility, geometry, kinematics, constraint, dynamics, stability, and fail-safe were met; however, this study is preliminary in nature.				
Title:	A memory coiling spiral as nucleus pulposus prosthesis: concept, specifications, bench testing, and first clinical results.			**Author(s)/ Reference:**	Husson JL, Korge A, Polard JL, Nydegger T, Kneubuhler S, Mayer HM. J Spinal Disord Tech. 2003 Aug; 16(4): 405-11.
Material:	Polycarbonate-urethane copolymer (thermoplastic elastomer) in the form of a "memory coiling spiral.	**Animal Model:**	Humans	**Implant Location:**	The intradiscal cavity left after a discectomy can be filled by a new nucleus prosthesis
Summary:	The intradiscal cavity left after a discectomy can be filled by a new nucleus prosthesis made of polycarbonate urethane in the form of a "memory coiling spiral." Biomechanical tests have demonstrated that this device compensates for the loss of disc height, decreases the compression of the facet joints, and restores the kinematics of the spinal segment, without deformation of the vertebral endplates or migration. The device is currently under clinical investigation. Inclusion and exclusion criteria of the pilot study are presented, and preliminary results of the first five patients supplied with the spiral are reported after an average follow-up time of 24 months. No migration of the device has been observed so far. With its easy application due to the standardized approach and the memory coiling mechanism, this device represents an advance within the nonfusion techniques.				
Title:	Beta-tricalcium phosphate as a substitute for autograft in interbody fusion cages in the canine lumbar spine.			**Author(s)/ Reference:**	Ohyama T, Kubo Y, Iwata H, Taki W. J Neurosurg. 2002 Oct; 97(3 Suppl): 350-4.
Material:	synthetic ceramic, beta-tricalcium phosphate (TCP), was examined as a substitute for autograft bone in a canine lumbar spine model.	**Animal Model:**	Dogs	**Implant Location:**	An interbody fusion cage has been introduced for cervical anterior interbody fusion.

Summary:	In this study a synthetic ceramic, beta-tricalcium phosphate (TCP), was examined as a substitute for autograft bone in a canine lumbar spine model. An interbody fusion cage has been introduced for cervical anterior interbody fusion. Autogenetic bone is packed into the cage to increase the rate of union between adjacent vertebral bodies. Good histological and biomechanical results were obtained for TCP-filled interbody fusion cages. The results were comparable with those obtained using autograft-filled cages, suggesting that there is no need to harvest iliac bone or to use allo- or xenografts to increase the interlocking strength between the cage and vertebral bone to achieve anterior cervical interbody fusion.				
Title:	A spiral implant as nucleus prosthesis in the lumbar spine.			**Author(s)/ Reference:**	Korge A, Nydegger T, Polard JL, Mayer HM, Husson JL. Eur Spine J. 2002 Oct; 11 Suppl 2: S149-53. Epub 2002 Aug 02.
Material:	polycarbonate urethane (Sulene PCU), and takes the form of a memory coiling spiral	**Animal Model:**	Humans	**Implant Location:**	Microdiscectomy represents the gold standard in disc surgery on the lumbar spine. The remaining defect in the intervertebral disc space can be filled with a newly developed nucleus prosthesis presented in this paper
Summary:	Microdiscectomy represents the gold standard in disc surgery on the lumbar spine. The remaining defect in the intervertebral disc space can be filled with a newly developed nucleus prosthesis presented in this paper. This prosthesis consists of polycarbonate urethane (Sulene PCU), and takes the form of a memory coiling spiral. It can be easily implanted using the standard microdiscectomy approach with no further tissue damage. Biomechanical tests have shown that anatomical distances can be restored by the spiral for both the facet joints and the endplates. Endplate deformations are not statistically different when compared to intact conditions. Inclusion and exclusion criteria of an *in vivo* pilot study are presented. The paper describes the insertion setup for the spiral and the technique of implantation. Five patients have been supplied with the implant to date. The first results on postoperative magnetic resonance images are presented.				
Title:	General principles of total disc replacement arthroplasty: seventeen cases in a nonhuman primate model			**Author(s)/ Reference:**	Cunningham BW, et al., Spine, 2003, 28(20), S118-S124.
Material:	AcroFlex and SB Charite	**Animal Model:**	Baboons	**Implant Location:**	Total disc replacement
Summary:	This study investigated the biomechanical, histochemical, and biologic ingrowth characteristics of two different lumbar disc prostheses-AcroFlex (DePuy-AcroMed) and the SB Charite (DePuy-AcroMed)-for total disc replacement arthroplasty in baboons. Conclusions showed that the porous ingrowth, percentage pore ingrowth coverage at the bone-metal interface was more favorable for total disc replacement compared to that reported for cementless total joint components in the appendicular skeleton (range 10-30%).				
Title:	Bone ingrowth fixation of artificial intervertebral disc consisting of bioceramic-coated three-dimensional fabric			**Author(s)/ Reference:**	Takahata M, et al., Spine 2003, 28(7), 637-644.
Material:	Bioceramic-coated (Apatite–wollastonite containing glass ceramic blocks) three-dimensional fabric (3-DF: FABRICUBE, Japan)	**Animal Model:**	Sheep	**Implant Location:**	Artificial disc

Summary:	The bone-bonding characteristic of a new artificial intervertebral disc consisting of bioceramic-coated three-dimensional fabric was evaluated mechanically and histologically in an *in vivo* sheep model. The findings showed that the three-dimensional fabric disc was firmly fixed to the vertebral body by bone ingrowth, and that this biologic fixation was preserved even under the spinal segmentally mobile condition.				
Title:	Analysis of porous ingrowth in intervertebral disc prostheses: a nonhuman primate model			**Author(s)/ Reference:**	McAfee PC, et al., Spine 2003, 28(4), 332-340.
Material:	Hydroxyapatite-coated SB Charite prosthesis	**Animal Model:**	Baboons	**Implant Location:**	Total disc prosthesis
Summary:	A study was conducted to investigate the biomechanical, histochemical, and biologic ingrowth characteristics of the most widely used total disc prosthesis, the hydroxyapatite-coated SB Charite prosthesis. Seven mature baboons (Papio cynocephalus) underwent L5-L6 total disc replacement through an anterior transperitoneal approach. porous ingrowth (percentage of pore ingrowth coverage at the bone-metal interface) was more favorable for total disc replacement than for cementless total joint components in the appendicular skeleton (range, 10-30%).				
Title:	Total disc replacement arthroplasty using the AcroFlex lumbar disc: a non-human primate model			**Author(s)/ Reference:**	Cunningham et al., European Spine Journal, 11, 2002, S115-S123.
Material:	AcroFlex lumbar disc	**Animal Model:**	Mature male baboons	**Implant Location:**	Intervertebral disc prosthesis
Summary:	Using a non-human primate model, this study was undertaken to investigate the efficacy of the AcroFlex lumbar disc as an intervertebral disc prosthesis, based on biomechanical, histopathologic and histomorphometric analyses. This project serves as the first comprehensive *in vivo* investigation into the AcroFlex disc prosthesis, and establishes a research model in the evaluation of total disc replacement arthroplasty.				
Title:	Artificial intervertebral disc replacement using bioactive three-dimensional fabric: design, development, and preliminary animal study			**Author(s)/ Reference:**	Kotani Y et al., Spine, 27(9), 2002, 929-935.
Material:	Triaxial 3D fabric woven with an ultra-high molecular weight polyethylene fire and spray coated bioactive ceramics on the disc surface	**Animal Model:**	Sheep	**Implant Location:**	Total intervertebral disc
Summary:	A new artificial intervertebral disc was developed, and its intrinsic biomechanical properties, bioactivity, and the effectiveness as a total disc replacement were evaluated in vitro and in vivo. The artificial intervertebral disc consists of a triaxial three-dimensional fabric (3-DF) woven with an ultra-high molecular weight polyethylene fiber, and spray-coated bioactive ceramics on the disc surface. The arrangement of weave properties was designed to produce mechanical behaviour nearly equivalent to the natural intervertebral disc. Total intervertebral disc replacement at L2-L3 and L4-L5 was performed using 3-DF disc with or without internal fixation in a sheep lumbar spine model. The segmental biomechanics and interface histology were evaluated after surgery at 4 and 6 months.				
Title:	Biomechanical and morphologic evaluation of a three-dimensional fabric sheep artificial intervertebral disc: in vitro and *in vivo* analysis			**Author(s)/ Reference:**	Kadoya K, et al., Spine, 2001, 26(14), 1562-1569.

Material:	Triaxial 3-DF woven from a specially designed fibre (a bundle of 50 ultra-high molecular weight polyethylene filaments) coated with linear low-density polyethylene to hold the individual filaments together. Corona discharge treatment was used to oxidize this fibre, enhancing its adhesive properties. To impart biologic activity, micropowders of unsintered hydroxyapatite or apatite-wollastonite glass ceramics were sprayed on the surface	**Animal Model:**	Sheep	**Implant Location:**	Artificial disc
Summary:	This study investigated the static, viscoelastic, and fatigue properties of a three-dimensional fabric disc in comparison with natural sheep disc and to evaluate their biomechanical and morphologic alteration *in vivo*. The sheep three-dimensional fabric disc exhibited biomechanical and morphologic biostability, appropriate viscoelasticity, and excellent fatigue properties. The three-dimensional fabric disc has a potential for clinical application of human intervertebral disc replacement.				
Title:	Real-time *in vivo* loading in the lumbar spine: part 1. Interbody implant: load cell design and preliminary results			**Author(s)/ Reference:**	Ledet EH, et al., Spine, 2000, 25(20), 2595-2600.
Material:	Strain gauges mounted on the surface of a carbon fibre box type implant to study force-strain relationship	**Animal Model:**	Baboons	**Implant Location:**	Instrumented interbody implants
Summary:	Instrumented interbody implants were placed into the disc space of a motion segment in two baboons. During the animal's activities, implants directly measured *in vivo* loads in the lumbar spine by telemetry transmitter. The objectives were to develop and test an interbody implant-load cell and use the implant to measure directly loads imposed on the lumbar spine of the baboon, a semiupright animal. The study concluded that the study technique and technology were efficacious for measuring real-time *in vivo* loads in the spine.				
Title:	Mechanical Evaluation Of A Canine Intervertebral Disc Spacer - In-Situ And In-Vivo Studies			**Author(s)/ Reference:**	Vuonohawkins M, et al., J. Orthopaedic Research, 1994, 12(1), 119-127.
Material:	Elastomeric spacer with hydroxyapatite ingrowth surfaces	**Animal** Model:	Dogs	**Implant Location:**	Intervertebral disc spacer
Summary:	An elastomeric intervertebral disc spacer with hydroxyapatite ingrowth surfaces was implanted in a canine model. The mechanical behavior of motion segments at time 0 and at 3, 6, and 12 months and the effect of the interface between the spacer and vertebral bone on implant stability and bone ingrowth were studied. All animals tolerated the surgery well and none had permanent neurological impairment. The measured parameters indicated that behavior of the spacer-motion segment composite appeared to return to normal within 3-6 months. However, despite use of a porous hydroxyapatite on the implant surface, there was no significant bone ingrowth. Instead, a layer of dense fibrous connective tissue was formed at the spacer-vertebral bone interface.				

ANNEX 4 – *IN VIVO* STUDIES OF SKIN SUBSTITUTE MATERIALS

Title:	*In vivo* mesenchymal cell recruitment by a scaffold loaded with transforming growth factor beta1 and the potential for in situ chondrogenesis			**Author(s)/ Reference:**	Huang et al., Tissue Eng. 8, 2003, 469-482
Material:	PCL/TGF-beta1-loaded fibril glue	**Animal Model:**	Rabbits	**Implant Location:**	Subcutaneous, Intramuscular,Subperiostial
Summary:	The objectives of this study were (1) to develop a biphasic implant made of a bioresorbable polymeric scaffold n combination with TGF-beat1-loaded fibrin glue for tissue-engineering applications, and (2) to determine whether the implant made of polycaprolactone (PCL) scaffold and TGF-beta1-loaded fibrin glue could recruit mesenchynmal cells and induce the process of cartilage formation when implanted in ectopic sites. Scaffold loaded with various does of TGF-beta1-fibrin glue were implanted into rabbits. Conclusions were postitive.				
Title:	Tissue-engineered cartilage by in vivo culturing of chondrocytes in PLGA –collagen hybrid sponge			**Author(s)/ Reference:**	Sato et al., Mat. Sci. and Eng.:C , 2003, 17 (1-2), 83-89
Material:	PLGA/ Collagen sponge	**Animal Model:**	Mice	**Implant Location:**	Subcutaneous
Summary:	Hybrid sponge of poly(DL-lactic-co-glycolic acid) (PLGA) and collagen was used as the porous scaffold with PLGA sponge and collagen sponge used as the controls. They were seeded with bovine chondrocytes (low and high densities) and implanted subcutaneously in the dorsum of athymic nude mice to tissue engineer articular cartilage in vivo. The PLGA –collagen hybrid sponge implants maintained their original shapes, as did the PLGA sponge, whereas the collagen sponge collapsed. The mechanically strong PLGA sponge functioned as a skeleton and prevented the embedded collagen sponge from collapsing. The implants were examined histochemically by haematoxylin and eosin staining, by safranin O/fast green staining, and immunohistochemically by anti-collagen type II antibody. The cartilaginous matrices were more homogeneously distributed in the PLGA –collagen hybrid sponge and collagen sponge than in the PLGA sponge.				
Title:	Studies on biodegradation and release of gentamicin sulphate from interpenetrating network hydrogels based on poly(acrylic acid) and gelatin: in vitro and *in vivo*			**Author(s)/ Reference:**	Changez M, et al., Biomaterials 2004, 25(1), 139-146.
Material:	Cylindrical-shaped hydrogel made of interpenetrating polymer network based on poly(acrylic acid) and gelatin	**Animal Model:**	Rats	**Implant Location:**	Subcutaneous, in the back
Summary:	Impenetrating network hydrogels (IPN's) based on poly(acrylic acid) and gelation (Ge) were evaluated for in vitro and *in vivo* biodegradation and *in vivo* release of gentamicin sulphate. Results showed that with the increase of acrylic acid content in the polymer, the rate of degradation decreases, and a reverse phenomenon was observed with increasing Ge content in the hydrogel.				
Title:	Photocrosslinkable chitosan hydrogel containing fibroblast growth factor-2 stimulates wound healing in healing-impaired db/db mice			**Author(s)/ Reference:**	Obara K, et al., Biomaterials 2003, 24(20), 3437-3444.
Material:	Photocrosslinkable chitosan insoluble flexible hydrogel including fibroblast growth factor-2	**Animal Model:**	Normal and diabetic mice	**Implant Location:**	Skin on back

Summary:	In order to evaluate the accelerating effect of ultraviolet irradiation to a photcrosslinkable chitosan (Az-CH-LA) aqueous solution on wound healing, full thickness skin incisions were made on the back of healing-impaired diabetic mice and their normal littermates.				
Title:	Induction of vascular endothelial growth factor by fibrin as a dermal substrate for cultured skin substitute			**Author(s)/ Reference:**	Hojo M, et al., Plastic & Reconstructive Surgery 2003, 111(5), 1638-1645.
Material:	Fibrin gel and type-I cultured skin substitute	**Animal Model:**	Mice	**Implant Location:**	Subcutaneous
Summary:	This study investigated the effect of fibrin glue gel as a dermal substrate for a cultured skin substitute, using human keratinocytes and dermal fibroblasts. A collagen-cultured skin substitute was also examined for comparison. Experiments were performed in vitro and on athymic mice. Conclusions were that the use of fibrin as a dermal substrate for cultured skin substitute increases the secretion of vascular endothelial growth factor (VEGF), improves regeneration of mature epidermal structure after *in vivo* transplantation, and promotes the migration of vascular endothelial cells.				
Title:	Long-term remodeling of a bilayered living human skin equivalent (Apligraf) grafted onto nude mice: immunolocalization of human cells and characterization of extracellular matrix			**Author(s)/ Reference:**	Guerret S, et al., Wound Repair & Regeneration 2003, 11(1), 35-45.
Material:	Apligraf®, living skin composed of bovine collagen lattice containing livin human fibroblasts overlaid with a fully differentiated epithelium made of human keratinocytes	**Animal Model:**	Mice	**Implant Location:**	Graft on central dorsum
Summary:	Apligraf, a bioengineered living skin, is composed of a bovine collagen lattice containing human fibroblasts overlaid with a fully differentiated epithelium made of human keratinocytes. To investigate its prpogressive remodelling, athymic mice were grafted and the cellular and extracellular matrix components studied. Authors observed that the tissue provided living and bioactive cells to the wound site up to one year after grafting.				
Title:	Nerve regeneration in a collagen-chitosan tissue-engineered skin transplanted on nude mice			**Author(s)/ Reference:**	Gingras M, et al., Biomaterials 2003, 24(9), 1653-1661.
Material:	Collagen-chitosan sponge seeded with human fibroblasts and keratinocytes	**Animal Model:**	Nude mice	**Implant Location:**	Deep wound created in the back up to the muscle
Summary:	A reconstructed skin made of a collagen-chitosan sponge seeded with human fibroblasts and keratincocytes and grown in vitro for 31 days was developed for the treatment of deep and extensive burns. The skin was transplanted onto the back of mice to assess whether it could promote nerve regeneration *in vivo*. The conclusions were that the 3-D architecture of the skin sponge encourages nerve growth.				
Title:	Influence of mesh materials on collagen deposition in a rat model			**Author(s)/ Reference:**	Junge K, et al., J. Investigative Surgery 2002, 15(6), 319-328.

Material:	Mesh materials including polyester, pure polypropylene and composite of polypropylene and polyglactin	**Animal Model:**	Rats	**Implant Location:**	Abdominal replacement
Summary:	Because of the widespread use of alloplastic meshes for the surgical repair of hernias, an animal study was performed to analyse the influence of various mesh materials on the quantity and quality of collagen deposition. An abdominal replacement was performed on rats using polyester (PE), a pure polypropylene (PP) and a composite mesh made of polypropylene and polyglactin (PG). The results of the study supported the notion that wound healing is affected by mesh implantation. The quality of the extracellular matrix (ECM) depostion is markedly influencec by the kind of mesh material.				
Title:	Effects of nitric oxide releasing poly(vinyl alcohol) hydrogel dressings on dermal wound healing in diabetic mice			**Author(s)/ Reference:**	Masters KSB, et al., Wound Repair & Regeneration 2002, 10(5), 286-294.
Material:	Polyvinyl alcohol hydrogel	**Animal Model:**	Genetically diabetic mice	**Implant Location:**	Dorsal skin
Summary:	Nitric oxide 9NO) has been proposed as a possible active agent for enhancing wound healing. This study examined the in vitro and *in vivo* responses to a novel hydrogel that produces therapeutic levels of NO. A hydrogel wound dressing was fabricated using ultraviolet light-initiated polymerisation from poly(vinyl alcohol) with a NO donor covalently coupled to the polymer backbone. The results of this study suggested that exogenous NO released from a hydrogel wound dressing has potential to modulate wound healing.				
Title:	Human dermal microvascular endothelial cells form vascular analogs in cultured skin substitutes after grafting to athymic mice			**Author(s)/ Reference:**	Supp DM, et al., Faseb Journal, 2002, 16(8), 797-804.
Material:	Dermal substitutes consisted of acellular collagen-glycosaminoglycan (GAG) substrates inoculated with fibroblasts or a mixture of fibroblasts and human dermal microvascular endothelial cells (HDMEC)	**Animal Model:**	Mice	**Implant Location:**	Wounds on flanks
Summary:	Cultured skin substitues (CSS) consisting of autologous fibroblasts and keratinocytes combined with biopolymers are an adjunctive treatment for large excised burns. CSS were prepared containing human keratinocytes, fibroblasts and dermal microvascular endothelial cells (HDMEC) isolated from a single skin sample. These were then grafted onto athymic mice. The results demonstrate HDMEC transplanation in a clinically relevant cultured skin model, persistence of HDMEC after grafting, and HDMEC organisation into vascular analogs in vitro and *in vivo*.				
Title:	Reconstructed human skin produced in vitro and grafted on athymic mice			**Author(s)/ Reference:**	Pouliot R, et al., Transplantation 2002, 73(11), 1751-1757.

Material:	Reconstructed human skin made by culturing newborn or adult keratinocytes on superimposed fibrous sheets obtained after culturing human fibrobasts with ascorbic acid	**Animal Model:**	Mice	**Implant Location:**	Dorsal muscular bed
Summary:	A reconstructed human skin (rHS) was made by culturing newborn or adult keratinocytes on superimposed fibrous sheets obtained after culturing human fibroblasts with ascorbic acid. Ten days after keratinocyte seeding, skins were grafted on athymic mice. Conclusions were that this new rHS model gives supple and easy to handle skins while demonstrating an adequate wound healing on mice.				
Title:	Collagen fibril network and elastic system remodeling in a reconstructed skin transplanted on nude mice			**Author(s)/ Reference:**	Berthod F, et al., Matrix Biology, 2001, 20(7), 463-473.
Material:	Collagen sponge seeded with human fibroblasts and keratinocytes	**Animal Model:**	Nude mice	**Implant Location:**	Back skin
Summary:	A reconstructed skin made of a collagen sponge seeded with human fibroblasts and keratinocytes was prepared and transplanted onto the back of mice to assess whether this reconstructed skin could prvent scar formation. The reconstructed skin model promoted in only 90 days the remodelling of an extracellular matrix similar to normal dermis.				
Title:	Strategies to improve the take of commercially available collagen/glycosaminoglycan wound repair material investigated in an animal model			**Author(s)/ Reference:**	Grant I, et al., Burns 2001, 27(7), 699-707.
Material:	Wounds were isolated by percutaneous PTFE chambers and grafted with Integra™ Artificial Skin, the wounds were closed using sutures and surrounded by protective jackets fashioned from thermoplastic Spectrum secured with Velcro® straps and padded with medium density furniture foam	**Animal Model:**	Pigs	**Implant Location:**	Wounds down to muscle fascia were made on the flank of each animal
Summary:	This study describes how the successful bio-integration of Integra (TM) Artificial Skin was accomplished in isolated full-thickness wounds in the pig, by the use of sequential protocol modifications that more intimately opposed the material to the wound bed. Further improvement was achieved by measures to reduce bacterial colonisation including the use of topical anti-microbial agents. Histological methods were used for wound analysis.				
Title:	Organotypical engineering of differentiated composite-skin equivalents of human keratinocytes in a collagen-GAG matrix (INTEGRA Artificial Skin) in a perfusion culture system			**Author(s)/ Reference:**	Kremer M, et al., Langenbecks Archives of Surgery 2001, 386(5), 357-363.

Material:	Human keratinocytes seeded into Integra Artificial Skin	**Animal Model:**	Mice	**Implant Location:**	Graft
Summary:	In this study, keratincocytes were seded onto INTEGRA artificial skin and placed in a perfusion culture system in order to evaluate the possibility of producing composite grafts in an automated system. These were grafted onto athymic mice to evaluate their potential to reconstitute a full-thickness skin substitute *in vivo*. Conclusions showed that engineering of differentiated composite skin equivalents is possible in a perfusion culture system.				
Title:	Effects of collagen matrix containing transforming growth factor (TGF)-beta(1) on wound contraction			**Author(s)/ Reference:**	Tateshita T, et al., J. Dermatological Science 2001, 27(2), 104-113.
Material:	composite was produced by mixing fibrillar collagen (FC) with helix content of the denatured atelocollagen used with/without transforming growth factor (TGF)-beta-1	**Animal Model:**	Rabbits	**Implant Location:**	Wounds
Summary:	This study evaluated the effectiveness of transforming growth factor (TGF)-beta1 on wound contraction, both alone and in combination with collagen matrix, using an *in vivo* delayed wound healing type model. The results showed that the application of TGF-beta1 treated collagen matrix is effective for preventing contraction producing so called “neodermis” in treating a delayed healing type model and may be highly beneficial for treating chronic wounds.				
Title:	*In vivo* cultured skin composed of two-layer collagen sponges with preconfluent cells			**Author(s)/ Reference:**	Morimoto N, et al., Annals of Plastic Surgery 2001, 47(1), 74-81.
Material:	Two layered collagen sponges with different pde sizes and crosslink densities, seeded with fibroblasts and keratinocytes	**Animal Model:**	Immunodeficient mice	**Implant Location:**	Wounds on backs
Summary:	In a previous study, the authors succeeded in producing cultured skin easily in a short period of time by layering two collagen sponges. In this study, they grafted the cell-preconfluent artificial skin immediately after seeding the cells Two collagen sponges were used with different pore sizes and crosslink densities. Two weeks after grafting, epithelium and dermislike tissue were formed. This cell-preconfluent artificial skin composed of two-layer collagen sponges seems promising for widespread clinical use.				
Title:	Srivastava A, DeSagun EZ, Jennings LJ, Sethi S, Phuangsab A, Hanumadass M, Reyes HM, Walter RJ			**Author(s)/ Reference:**	Srivastava A, et al., Annals of Surgery 2001, 233(3), 400-408.
Material:	Xenogenic (porcine) and allogenic acellular dermal matrix were produced by treating porcine or rat skin with Dispase and Triton X-100	**Animal Model:**	Rats	**Implant Location:**	Dorsum

Summary:	The objective of this study was to examine porcine acellular dermal matrix (ADM) as a xenogenic dermal substitute in a rat model. Xenogenic and allogenic ADM's were produced by treating porcine or rat skin with Dispase and Triton X-100 and implanted into rats. Conclusions were that Dispase-Triton-treated allogenic ADM was useful as a dermal substitute in full-thickness skin defects, but healing with xenogenic ADM was poor.				
Title:	Effects of a collagen matrix containing prostaglandin E(1) on wound contraction			**Author(s)/ Reference:**	Ono I, et al., J. Dermatological Science 2001, 25(2), 106-115.
Material:	Composite collagen sponge made with fibrillar collagen and helix content of the denatured atelocollagen with/without prostaglandin (PG) E-1	**Animal Model:**	Rabbits	**Implant Location:**	Wounds on back
Summary:	In this study, the effectiveness of prostaglandin (PG) E(1) in inhibiting wound contraction, both alone and in combination with collagen matrix, using an *in vivo* full thickness skin defect model. It was concluded that collagen matrix combined with PG E(1) is more effective for preventing contracture producing so called neodermis than collagen matrix alone.				
Title:	Reduction of abdominal adhesions using composite collagen-GAG implants for ventral hernia repair			**Author(s)/ Reference:**	Butler CE, et al., J. Biomedical Materials Research 2001, 58(1), 75-80.
Material:	Polypropylene mesh within a porous collagen-glycosaminoglycan matrix	**Animal Model:**	Guinea pigs	**Implant Location:**	Surgically created ventral hernia (peritoneal cavity)
Summary:	Composite implants were created by interposing polypropylene (PP) mesh within a porous glycosaminoglycan (CG) matrix-created composite implant. The implants were cross-linked with glutaraldehyde one group (CG-G/PP) or left untreated (CG-nG/PP). Integrating a biodegradable extracellular matrix analog with a permanent structural biomaterial reduced adhesions in the animal model.				
Title:	Influence of transforming growth factor-beta3 on fibrous capsule formation around microgrooved subcutaneous implants *in vivo*			**Author(s)/ Reference:**	Gehrke TAE, et al., Tissue Eng., 2000, 6(5), 505-517.
Material:	Silicone implants with microgrooves	**Animal Model:**	Guinea pigs	**Implant Location:**	Subcutaneously in the back
Summary:	This study examined whether transforming growth factor-beta3 (TGF-beta3) can influence the organisation of a fibrous capsule around implants *in vivo*. Silicone implants with microgroves were loaded with human reombinant TGF-beta3. The implants were inserted subcutaneously in the backs of guinea pigs. There were no significant differences seen between the TGF-beta3-coated implants and controls.				
Title:	Biocompatibility of electroactive polymers in tissues			**Author(s)/ Reference:**	Kamalesh S, et al., J. Biomedical Materials Research 2000, 52(3), 467-478.

Material:	Ethylene-vinyl acetate copolymer, polyethylene and polyaniline films in the emeraldine nigraniline and leucomeraldine intrinsic oxidation states	**Animal Model:**	Rats	**Implant Location:**	Beneath dorsal skin
Summary:	The biocompatibility of ethylene-vinyl acetate copolymer (EVAc), polyethylene (PE), and polyanilie (PANi) films in the emaraldine (EM), nigraniline (NA) and leucoemeraldine (LM) intrinsic oxidation states were assessed through subcutaneous implantation into rats. The polymers were not found to provoke any inflammatory responses near the implantation.				
Title:	Evaluation of dermal-epidermal skin equivalents ('composite-skin') of human keratinocytes in a collagen-glycosaminoglycan matrix(Integra artificial skin).			**Author(s)/ Reference:**	Kremer M, et al., British J. Plastic Surgery, 2000, 53(6), 459-465.
Material:	Human keratinocytes in homogeneous distribution and depth into Integra ™	**Animal Model:**	Mice	**Implant Location:**	Skin graft
Summary:	The goal of this experimental study was to develop a method to integrate human keratinocytes in homogenous distribution and depth into Integra Artificial Skin. The seeded cell-matrix composites were grafted onto athymic mice in order to evaluate their potential to reconstitute a human epidermis *in vivo*. Researchers were able to demonstrate that the inoculated human keratinocytes reproducibly displayed a homogenous pattern of distribution, adherence, proliferation and confluence.				
Title:	Two-step grafting of the full thickness skin defects in pigs using the composite of atelocollagen and hyaluronic acid			**Author(s)/ Reference:**	Brychta P, et al., Acta Veterinaria BRNO 2000, 69(2), 123.
Material:	Composite of bovine atelocollagen and hyaluronic acid implanted as the dermal substitute followed by a thin epidermal autograft after 10 days	**Animal Model:**	Pigs	**Implant Location:**	Wounds
Summary:	The composite of bovine atelocollagen and hyaluronic acid (HyproDerm) was implanted as the dermal substitute in pig wounds in the first step. The overgrafting of this composite with a thin epidermal autograft followed as the second step ten days later. Planimetric, histologic and clinical evaluations (using the Vancouver Scar Score) of healed wounds were carried out. Studied wounds were compared with those left for spontaneous healing and those grafted immediately using dermoepidermal graft without any dermal substitute. The HyproDerm reduced the shrinkage of resurfaced wounds and improved the quality of reconstructed skin in pigs.				
Title:	Creation of an acellular dermal matrix from frozen skin			**Author(s)/ Reference:**	Mizuno H, et al., Aesthetic Plastic Surgery 1999, 23(5), 316-322.
Material:	Acellular dermal matrix prepared from normal human frozen skin	**Animal Model:**	Nude rats	**Implant Location:**	Skin graft
Summary:	In this study, a simple method of processing frozen surplus skin to produce an acellular, strucurally intact, dermal matrix. Using an animal model, the acellular matrix prepared from normal human skin was transplanted into full-thickness wounds on rats. It was seen that the dermal matrix supported fibroblast infiltration and neovascularisation.				

Title:	Effects of a collagen matrix containing basic fibroblast growth factor on wound contraction			**Author(s)/ Reference:**	Ono I, et al., J. Biomedical Materials Research 1999, 48(5), 621-630.
Material:	Composite collagen sponge made with fibrillar collagen and helix content of the denatured atelocollagen with/without basic fibroblast growth factor (bFGF)	**Animal Model:**	Rabbits	**Implant Location:**	Wounds
Summary:	The effectiveness of basic fibroblast growth factor (bFGF) in inhibiting wound contraction, both alone and in combination with collagen matrix, was evaluated using a simulated *in vivo* delayed healing type model. It was seen that application of bFGF-treated collagen matrix to chronic wounds such as decubitus, and diabetic and leg ulcers may be prove to be highly beneficial in clinical practice.				
Title:				**Author(s)/ Reference:**	Chen F, et al., Urology 1999, 54(3), 407-410.
Material:	Acellular collagen matrix obtained and processed from porcine bladder submucosa	**Animal Model:**	Rabbits	**Implant Location:**	Ventral urethral defect
Summary:					
Title:	Xenogeneic acellular dermal matrix as a dermal substitute in rats			**Author(s)/ Reference:**	Srivastava A, et al., J. Burn Care & Rehabilitation 1999, 20(5), 382-390.
Material:	Xenogenic (porcine) and allogenic acellular dermal matrix prepared by using Dispase II and Triton X-100	**Animal Model:**	Rats	**Implant Location:**	Dorsum graft
Summary:	Xenogenic acellular dermal matrix (ADM) was prepared from commercially available, cryopreserved porcine skin with the use of Dispase II and Triton X-100. Full-thickness injuries were created in the dorsum of each rat and grafts applied. Graft take was poor in the wounds that contained xenogenic ADM at 14 days after surgery and moderately good in this that contained allogenic ADM				
Title:	A modified culture system for epidermal cells for grafting purposes: an in vitro and *in vivo* study			**Author(s)/ Reference:**	Van Dorp AGM, et al. Wound Repair & Regeneration 1999, 7(4), 214-225.
Material:	Human or porcine epidermal keratinocytes on polyester filter substrate	**Animal Model:**	Pigs	**Implant Location:**	Skin wounds
Summary:	A fully differentiated epithelium mimicking the features of native epidermis was obtained in vitro by culturing human or porcine epidermal keratinoytes on polyester filter substrate. When grafted onto full-thickness skin wounds in pigs, the take of cell sheets detached from the filter with dispase was significantly higher in comparison to mechanically detached keratinocytes.				
Title:	Promising therapy for congenital giant pigmented nevi using acellular autograft nevi-dermal matrix			**Author(s)/ Reference:**	Mizuno H, et al., Annals of Plastic Surgery 1999, 43(3), 273-282.

Material:	Processed acellular nevi-dermal matrix from frozen skin	**Animal Model:**	Nude rats	**Implant Location:**	Full-thickness skin defects
Summary:	In this study, the authors investigated the potential use of an acellular autograft nevi-dermal matrix in combination with a split-thickness skin graft. To address whether the processed acellular nevi-dermal matrix from frozen skin could be reconstitutes as a viable dermal base, the authors grafted it onto full-thickness skin defects in rats. Fibroblast and infiltration and neovascularisation into the acellular nevi-dermal matrix were observed.				
Title:	Use of a collagen/elastin-membrane for the tissue engineering of dermis			**Author(s)/ Reference:**	Hafemann B, et al., Burns 1999, 25(5), 373-384.
Material:	Xenogenic membranes consisting of processed native collagen and elastin with in vitro-cultured autogenic keratinocyts	**Animal Model:**	Rats	**Implant Location:**	Graft
Summary:	In an experimental model in rats, xenogenic membranes consisting of processed native collagen and elastin were grafted to serve as a template for the formation of a neo-dermis. The process of tissue reconstruction and the digestion of the grafted membrane components were analysed by histological and immunohistochemical methods as well as electron microscopy. It was seen that the three-dimensional matrix structure offers a promising scaffold for a tissue engineering strategy to restore skin structure and function.				
Title:	The allogeneic response to cultured human skin equivalent in the hu-PBL-SCID mouse model of skin rejection			**Author(s)/ Reference:**	Briscoe DM, et al., Transplantation 1999, 67(12), 1590-1599.
Material:	Allogenic human skin equivalent (Apligraf)	**Animal Model:**	Humanised SCID mice	**Implant Location:**	Graft
Summary:	To determine the basis for the rejection of engineered tissues, the immune response to allogenic human skin equivalent (HSE, also called Apligraf) was analysed. In one model, human skin or HSE was transplanted onto humanised mice so that graft survival could be analysed. Both human foreskin and HSE successfully engrafted onto mice and remained stable for over 6 months.				
Title:	In-vivo tissue response to polyaniline			**Author(s)/ Reference:**	Wang CH, et al., Synthetic Metals 1999, 102(1-3), 1313-1314.
Material:	Polyaniline powders and films of the emeraldine nigraniline and leucoemeraldineand and surface modified emeraldine base films with acrylic acid and subsequent covalent immobilisation of collagen	**Animal Model:**	Rats	**Implant Location:**	Subcutaneous, below dorsal skin
Summary:	Polyaniline (PANi) powders and films of the emeraldine (EM), nigraniline (NA) and leucoemeraldine (LM) states, as well as surface-modified EM base films from graft copolymerization with acrylic acid (AAc) and the subsequent covalent immobilization of collagen were implanted into rats beneath the dorsal skin. It was observed that inflammation associated with the various forms of PANi was minimal after 50 weeks. Histological examinations of tissues revealed that the pristine EM film was encapsulated by several loosely arranged fibrous tissue. The collagen-immobilized EM film, on the other hand, showed no features resulting from tissue incompatibility near the implant.				

Title:	Denucleation promotes neovascularization of ePTFE *in vivo*			**Author(s)/ Reference:**	Boswell CA, et al., J. Biomedical Science - Polymer Edition 1999, 10(3), 319-329.
Material:	Expanded polytetrafluoroethylene altered by denucleation (removal of trapped air)	**Animal Model:**	Rats	**Implant Location:**	Subcutaneous and epididymal fat sites
Summary:	In this study, the healing characteristics of expanded polytetrafluoroethylene (ePTFE) were altered by denucleation, a process which removes the air trapped within the interstices of the material. After 5 weeks implanted into fat sites of rats, the material was explanted and the healing around the implant evaluated histologically. The results suggested that the presence of air nuclei within porous materials may contribute to the inappropriate healing response associated with these materials.				
Title:	Study on gelatin-containing artificial skin: I. Preparation and characteristics of novel gelatin-alginate sponge			**Author(s)/ Reference:**	Choi YS, et al., Biomaterials 1999, 20(5), 409-417.
Material:	Absorbable sponge composed of gelatin and alginate	**Animal Model:**	Rats	**Implant Location:**	Wound
Summary:	An absorbable sponge composed of gelatin and alginate was prepared by a new crosslinking method that improved the efficiency of crosslinking. An *in vivo* test was employed to confirm the applicability of this gelatin-alginate sponge as a wound dressing material. The rats showed a good wound healing effect.				
Title:	Organized skin structure is regenerated *in vivo* from collagen-GAG matrices seeded with autologous keratinocytes			**Author(s)/ Reference:**	Compton CC, et al., J. Investigative Dermatology 1998, 110(6), 908-916.
Material:	Collagen-glycosaminoglycan matrix seeded with autologous keratinocytes	**Animal Model:**	Pigs	**Implant Location:**	Full-thickness wounds
Summary:	A well-characterized collagen-glycosaminoglycan matrix (CGM) that has been shown to function as a dermal analog was seeded with freshly disaggregated autologous keratinocytes and applied to full-thickness wounds in a porcine model. this study, the temporal sequence of events in epidermal and neodermal formation was analyzed histopathologically and immunohistochemically from 4 to 35 d post-grafting. Within 1 mo, many structural components of normal skin were reconstituted.				
Title:	Living skin substitutes: survival and function of fibroblasts seeded in a dermal substitute in experimental wounds			**Author(s)/ Reference:**	Lamme EN, et al., J. Investigative Dermatology 1998, 111(6), 989-995.
Material:	Artificial elastin/collagen dermal subtitute seeded with fibroblasts	**Animal Model:**	Pigs	**Implant Location:**	Full-thickness wounds
Summary:	This study investigated the fate of fibroblasts seeded in an artificial elastin/collagen dermal substitute and the influence of the seeded fibroblasts on cell migration and dermal substitute degradation after transplantation to experimental full-thickness wounds in pigs. Wounds were treated with either dermal substitutes seeded with autologous fibroblasts or acellular substitutes. The observed effects of seeded fibroblasts on dermal regeneration appeared to be mediated by reducing subcutaneous fibroblastic cell migration and/or proliferation into the wounds without impairing migration of monocytes/macrophages and endothelial cells. Moreover, the degradation of the implanted dermal substitute was retarded, indicating a protective activity of the seeded fibroblasts.				

Title:	Glucocorticoid dynamics and impaired wound healing in diabetes mellitus			**Author(s)/ Reference:**	Bitar MS, American J. Pathology 1998, 152(2), 547-554.
Material:	Polyvinyl alcohol sponge disks	**Animal Model:**	Rats with diabetes mellitus	**Implant Location:**	Wound
Summary:	The aim of this study was to examine corticosterone dynamics and its role in the pathogenesis of impaired wound healing in diabetes mellitus (DM). The streptozotocin-treated rat was used as an animal model for type I DM. A linear skin incision and subcutaneously implanted polyvinyl alcohol sponge disks were considered as wound-healing models. Overall, the data provided evidence that the diabetic state is associated with hypercortisolemia and that this phenomenon may contribute to impaired wound healing in DM.				
Title:	Chemical inactivators as sterilization agents for bovine collagen materials			**Author(s)/ Reference:**	Doillon CJ, et al., J. Biomedical Materials Research 1997, 37(2), 212-221.
Material:	Bovine collagen sponge discs treated with either formic acid, tifluoroacetic acid, tetrafluoroethanol or hexafluoroisopropanol	**Animal Model:**	Mice	**Implant Location:**	Subcutaneous in the back
Summary:	The physicochemical changes and the in vitro and *in vivo* biocompatibility of collagen treated by formic acid (FA), trifluoroacetic acid (TFA), tetrafluorethanol (TFE), and hexafluoroisopropanol (HFIP) were investigated. In addition, the effects of these treatments on nucleic acids incorporated in collagen were analyzed. *in vivo*, implants induced a temporary inflammatory reaction that was prolonged with TFA and HFIP treatments. TFE and FA-treated collagen were thoroughly infiltrated by fibroblasts.				
Title:	Differences in dermal analogs influence subsequent pigmentation, epidermal differentiation, basement membrane, and rete ridge formation of transplanted composite skin grafts			**Author(s)/ Reference:**	Medalie DA, et al., Transplantation 1997, 64(3), 454-465.
Material:	Keratinocytes seeded onto both aceelular human dermis and fibroblast-contracted collagen gels	**Animal Model:**	Mice	**Implant Location:**	Graft
Summary:	This study evaluated the in vitro and *in vivo* function of composite skin equivalents based on two different dermal analogs. Keratinocytes derived from the same dark-skinned neonatal foreskins were seeded onto both acellular human dermis and fibroblast-contracted collagen gels. When transplanted to athymic mice, both composite grafts formed a fully differentiated human epidermis				
Title:	Clinical and histologic behavior of exposed porous high-density polyethylene implants			**Author(s)/ Reference:**	Sclafani AP, et al., Plastic & Reconstructive Surgery 1997, 99(1), 41-50.
Material:	Porous high-density polyethylene and silicone disks	**Animal Model:**	Rats	**Implant Location:**	Underneath doral skin
Summary:	In order to further investigate the response of porous high-density polyethylene under adverse conditions, three porous high-density polyethylene disks and one silicone disk were implanted underneath the dorsal skin in each of 12 rats. These alloplasts tolerated exposure well once host fibrovascular tissue had invaded the implant pores.				

Title:	Behavior of collagen-GAG matrices as dermal replacement in rodent and porcine models			**Author(s)/ Reference:**	Orgill DP, et al., Wounds - A Compendium of Clinical Research and Practice 1996, 8(5), 151-157.
Material:	Collagen-glycosaminoglycan copolymers	**Animal Model:**	Guinea pigs and Yorkshire pigs	**Implant Location:**	Full-thickness wounds
Summary:	The behavior of a highly specific and active collagen-glycosaminoglycan (CG) copolymer was evaluated in full-thickness wounds of Hartley guinea pigs and Yorkshire pigs. The results showed significant differences in wound contraction, cellular infiltration, and matrix degradation between the two models. Wound contraction, measured as a percent of original wound area, was delayed by the CG matrix in both models compared to full-thickness control wounds. CG matrices seem to alter the kinetics of wound closure in both the guinea pig model, which heals full-thickness dermal wounds primarily by contraction, and the porcine model, which heals primarily by epithelialization. Histologic observations showed both cellular infiltration and matrix degradation to occur more rapidly in the porcine model.				
Title:	Preformation of microvascular composite free flaps in the rat as an animal model			**Author(s)/ Reference:**	Steinhat H, et al., European Archives of Oto-Rhino-Laryngology 1996, 253(6), 325-328.
Material:	Porous polyethylene or titanium mesh implants in different forms and sizes as a "back side" to prepare skin flaps	**Animal Model:**	Rats	**Implant Location:**	Epigastric skin
Summary:	The design of microsurgically reanastomosed composite skin flaps, using porous polyethylene or titanium mesh implants as a "back side" in an animal model has been described. Two to 6 weeks after this procedure the flaps were lifted, transposed to the other side and the epigastric vessels were reanastomosed microsurgically. Eighty-three per cent of the skin flaps containing the titanium implants survived when the transplantation occurred 2 weeks after insertion of the implants. Concurrently all flaps with the implanted porous polyethylene (1 mm thick) showed signs of skin necrosis, especially concave-shaped implants. Histopathological evaluation of the titanium flaps showed a thin capsule around the implants and a minimal inflammatory reaction. All porous polyethylene implants resulted in a pronounced chronic infection. Transplantation of flaps containing perforated metals (such as the titanium mesh) was possible 2 weeks after insertion of the implant, whereas neovascularization of flaps with porous material required more than 4 weeks growth in situ to ensure at least a 50% viability of the skin.				
Title:	Evaluation of human skin reconstituted from composite grafts of cultured keratinocytes and human acellular dermis transplanted to athymic mice			**Author(s)/ Reference:**	Medalie DA, et al., J. Investigative Dermatology 1996, 107(1), 121-127.
Material:	Cultured human keratinocytes and de-epidermalised, acellular human dermis	**Animal Model:**	Mice	**Implant Location:**	Full-thickness wounds
Summary:	This study evaluated the use of composite grafts of cultured human keratinocytes and de-epidermalized, acellular human dermis to close full-thickness wounds in athymic mice. Grafts were transplanted onto athymic mice and studied up to 8 wk. Graft take was excellent, with no instances of infection or graft loss. The study demonstrated that composite grafts of cultured keratinocytes combined with acellular dermis are a useful approach for the closure of full-thickness wounds.				
Title:	Recent advances in tissue synthesis *in vivo* by use of collagen-glycosaminoglycan copolymers			**Author(s)/ Reference:**	Ellis DL, et al., Biomaterials 1996, 17(3), 291-299.
Material:	Collagen-glycoaminoglycan matices	**Animal Model:**	Guinea pigs	**Implant Location:**	Graft

Summary:	Biologically active analogues of the extracellular matrix (ECM) are synthesized by grafting glycosaminoglycan (GAG) chains onto type I collagen, and by controlling the physicochemical properties of the resulting graft copolymer. Collagen-GAG ECM analogues have previously been shown to induce regeneration of the dermis in humans and the guinea pig, and of the rat sciatic nerve. Current studies have emphasized elucidation of the molecular mechanism through which tissue-specific ECM analogues induce regeneration				
Title:	Evaluation of an allogeneic cultured dermal substitute composed of fibroblasts within a spongy collagen matrix as a wound dressing			**Author(s)/ Reference:**	Yamada N, et al., Scandinavian J. Plastic & Reconstructive Surgery & Hand Surgery 1995, 29(3), 211-219.
Material:	Cultured dermal substitute prepared by plating fibroblasts on to a spongy collagen matrix	**Animal Model:**	Rats	**Implant Location:**	Full-thickness skin defect on the dorsum
Summary:	The purpose of this study was to examine whether epithelialisation is promoted when an allogeneic cultured dermal substitute is used as a biological wound dressing. The dermal substitute was prepared by plating fibroblasts on to a spongy collagen matrix, and then culturing them for 7 to 10 days. The conclusions were that the application of this new fibroblastic cultured dermal substitute provided a good environment for the promotion of wound healing.				
Title:	Transplanted acellular allograft dermal matrix. Potential as a template for the reconstruction of viable dermis			**Author(s)/ Reference:**	Livesey SA, et al., Transplantation 1995, 60(1), 1-9.
Material:	Porcine skin processed to form an acellular, structurally intact, dermal matrix	**Animal Model:**	Rats	**Implant Location:**	Subcutaneous implant
Summary:	In this study, authors have investigated a method of processing porcine skin to produce an acellular, structurally intact, dermal matrix. They developed a process that de-epidermizes and decellularizes fresh porcine skin, while maintaining the basement membrane complex and the extracellular matrix structure of the dermis. The results suggest that skin processed by this method has the potential to be used as a permanent dermal allograft to augment the performance of a meshed split-thickness autograft (STSG) in the closure of full-thickness wounds				
Title:	Genetically modified skin to treat disease: potential and limitations			**Author(s)/ Reference:**	Krueger GG, et al., J. Investigative Dermatology 1994, 103(5), S76-S84.
Material:	Matrix of woven nylon housing a fibroblast generated collage and dead dermis used to deliver genetically modified human fibroblasts	**Animal Model:**	Rodents	**Implant Location:**	Graft
Summary:	Experience with transplanting cellular elements of skin or skin substitutes (defined as skin that possess the cell types and a dermal structure to develop into a functioning skin) to athymic rodents is considerable and is seen as a system where these questions can be answered. This paper reviews these questions and presents early analysis of genetically modified cells in skin substitutes *in vivo* and in vitro				
Title:	Ultrastructural features of composite skin cultures grafted onto athymic mice			**Author(s)/ Reference:**	Nolte CJM, et al., J. Anatomy 1994, 185, 325-333.

Material:	Composite skin cultures (Graftskin, LSE) consisting of epidermal keratinocytes seeded on a fibroblast containingcollagen matrix maintained at an air-liquid interface	**Animal Model:**	Mice	**Implant Location:**	Full-thickness wounds
Summary:	In this study, LSE were grafted onto full thickness wounds in athymic mice. Skin cultures (Graftskin, LSE), consist of epidermal keratinocytes seeded on a fibroblast-containing collagen matrix and maintained at the air-liquid interface. The grafted LSE integrated well into the host tissue and remained intact throughout the 60 d study period.				
Title:	Biocompatibility of a biodegradable matrix used as a skin substitute: an *in vivo* evaluation			**Author(s)/ Reference:**	Beumer GJ, et al., J. Biomedical Materials Research 1994, 28(5), 545-552.
Material:	Polymeric matrices with a dense top layer and porous under-layer, made of a polyether/polyester copolymer called Polyactive ™ and poly-L-lactide	**Animal Model:**	Rats	**Implant Location:**	Subcutaneously representing large body surface areas
Summary:	Synthetic biodegradable polymeric matrices, with a dense top layer and porous under-layer, made of a (poly)ether/(poly)ester (PEO:PBT) copolymer called Polyactive, and also of poly-L-lactide (PLLA), were investigated as part of a cell-seeded skin substitute for third-degree, large-scale skin defects. The biocompatibility of subcutaneously implanted matrices representing large body surface areas, were studied at 2, 4, 13, 26, and 52 weeks in rats. All matrices showed neovascular and fibrous tissue ingrowth into the porous underlayer within 2-4 weeks after implantation.				
Title:	Ultraviolet-A induced delayed wound contraction and decreased collagen content in healing wounds and implant capsules			**Author(s)/ Reference:**	Ozcan G, et al., Plastic & Reconstructive Surgery 1993, 92(3), 480-484.
Material:	Silicone cubes	**Animal Model:**	Rats	**Implant Location:**	Subcutaneously in irradiated area
Summary:	Chronic exposure to ultraviolet-A radiation causes changes in the biochemistry of dermal connective tissue. To investigate its effects on wound healing, Sprague-Dawley rats were irradiated for 4 months using a black ray ultraviolet-A radiation source (560 J/cm2). Full thickness skin wounds of 2 cm in diameter were then created on the back of each animal and silicone cubes were implanted subcutaneously into the irradiated area of the back.				
Title:	Inhibition of calcification *in vivo* by acyl azide cross-linking of a collagen-glycosaminoglycan sponge			**Author(s)/ Reference:**	Anselme K, et al., Matrix 1992, 12(4), 264-273.
Material:	Collagen-glycosaminoglycan sponge	**Animal Model:**	Rats	**Implant Location:**	Subcutaneous
Summary:	The biocompatibility of a collagen-glycosaminoglycan sponge composed of collagen (80%), chondroitin-4-sulfate (13.3%) and heparan sulfate (6.6%) was studied up to 3 months after subcutaneous implantation in rats by analysing cellular responses and calcification by histological and ultrastructural methods.				

Title:	In vitro production and subsequent transplantation of a living skin substitute in rat model			**Author(s)/ Reference:**	Ross Uh, European Archives of Oto-Rhino-Laryngology 1992, 249(5), 263-267.
Material:	Lyophilised human dura mater or lyophilised porcine dermis or a lamella of dehydrated human fascia lata all seeded with epidermal cells	**Animal Model:**	Rats	**Implant Location:**	Full-thickness skin defects
Summary:	Three different transplants were generated for in vitro production of a living skin substitute in a rat animal model. In all cases epithelial cells from a small skin biopsy were grown in cell culture. Compound 1 consisted of cells outgrowing on lyophilized human dura mater. Compound 2 was fabricated with lyophilized porcine dermis. In compound 3 a lamella of dehydrated human fascia lata was seeded with epidermal cells. These skin substitutes were used to cover full-thickness skin defects.				
Title:	Effects of platelet activating factor (PAF) and other vasoconstrictors on a model of angiogenesis in the mouse			**Author(s)/ Reference:**	Andrade SP, et al., International J. Experimental Pathology 1992, 73(4), 503-513.
Material:	Glycosaminoglycan sponge and Xe-133 washout technique	**Animal Model:**	Mice	**Implant Location:**	Graft
Summary:	The combination of sponge implant and 133Xe washout technique described in this paper provides a model to study neovascularization in mice which can be observed over several days in the same animal.				
Title:	Physical and biological properties of a new synthetic amino acid copolymer used as wound dressing			**Author(s)/ Reference:**	Eloy R, et al., J. Biomedical Materials Research 1992, 26(6), 695-712.
Material:	Synthetic amino acid copolymer	**Animal Model:**	Guinea pigs and rats	**Implant Location:**	Full-thickness skin defects and subcutaneously
Summary:	A new synthetic amino acid copolymer has been evaluated as wound covering. *In vivo* experiments were designed to provide qualitative and quantitative evaluation on its possible use as a skin substitute in full-thickness skin excision in the guinea pig. The results demonstrate that the membrane is not biodegradable.				
Title:	Reconstitution of the histologic characteristics of a giant congenital nevomelanocytic nevus employing the athymic mouse and a cultured skin substitute			**Author(s)/ Reference:**	Cooper ML, et al., J. Investigative Dermatology 1991, 97(4), 649-658.
Material:	Collagen-glycoaminoglycan matrix followed by placement of epidermal cells onto the opposite, laminated side of the matrix	**Animal Model:**	Mice	**Implant Location:**	Full-thickness wounds
Summary:	This study addresses the development of an animal model for human giant congenital nevomelanocytic nevi (GCNN). Skin grafts were made from 1) non-involved split-thickness skin from a 12-month-old GCNN patient, 2) nevus split-thickness skin from the same GCNN patient, 3) nevus full-thickness skin, and 4) cadaveric human split-thickness skin.				

Title:	*In vivo* optimization of a living dermal substitute employing cultured human fibroblasts on a biodegradable polyglycolic acid or polyglactin mesh			**Author(s)/ Reference:**	Meshes of polyglycolic acid and polyglactin-910 as carriers of cultured human fibroblasts
Material:	Meshes of polyglycolic acid and polyglactin-910 as carriers of cultured human fibroblasts	**Animal Model:**	Mice	**Implant Location:**	Grafts
Summary:	The design of a skin-substitute must address the need for a dermal component, as this mesenchymally-derived tissue is important in maintaining the integrity and function of skin. An *in vivo* study was undertaken to assess the use of two biodegradable meshes, polyglycolic acid and polyglactin-910, as carriers for cultured human fibroblasts in a living dermal replacement. The consistent vascularization and epithelialization of these grafts placed on athymic mice showed that this has potential in re-creating the dermis in a skin-substitute.				

ANNEX 5 – RECENT REVIEWS

BONE AND BONE SUBSTITUTES

- **Modern issues in bone graft substitutes and advances in bone tissue technology,** Sammarco VJ, Chang L ,Foot Ankle Clin. 2002 Mar;7(1):19-41
- **Bone graft substitutes,** McAuliffe JA, J Hand Ther. 2003 Apr-Jun;16(2):180-7
- **Bone substitutes in 2003: an overview,** Delloye C, Cnockaert N, Cornu O, Acta Orthop Belg. 2003;69(1):1-8
- **Bone regeneration graft materials,** Hoexter DL, J Oral Implantol. 2002;28(6):290-4
- **Bone tissue engineering: hope vs hype,** Rose FR, Oreffo RO, Biochem Biophys Res Commun. 2002 Mar 22;292(1):1-7
- **Bone healing and bone substitutes,** Costantino PD, Hiltzik D, Govindaraj S, Moche J, Facial Plast Surg. 2002 Feb;18(1):13-26

CARTILAGE

- **Engineering cartilage growth and development,** Kaufmnan MR, Tobias GW**,** Clin Plast Surg. 2003 Oct; 30(4): 539-46.
- **Tissue engineering of cartilage,** Randolph MA, Anseth K, Yaremchuk MJ**.** Clin Plast Surg. 2003 Oct; 30(4): 519-37.
- **Current state of cartilage tissue engineering,** Tuli R, Li WJ, Tuan RS**.** Arthritis Res Ther. 2003; 5(5): 235-8. Epub 2003 Aug 08.
- **Tissue-engineered versus native cartilage: linkage between cellular mechano-transduction and biomechanical properties,** Lee JH, Kisiday J, Grodzinsky AJ. Novartis Found Symp. 2003; 249: 52-64; discussion 64-9, 170-4, 239-41.
- **Articular cartilage bioreactors and bioprocesses,** Darling EM, Athanasiou KA**.** Tissue Eng. 2003 Feb; 9(1): 9-26.

INTERVERTEBRAL DISCS

- **Intervertebral disc prostheses**, Guyer RD, Ohnmeiss DD**.** Spine. 2003 Aug 1; 28(15): S15-23.

- **Total disc replacement for chronic low back pain: background and a systematic review of the literature:** de Kleuver M, Oner FC, Jacobs WC. Eur Spine J. 2003 Apr; 12(2): 108-16. Epub 2002 Dec 07.

- **The artificial disc: theory, design and materials:** Bao QB, McCullen GM, Higham PA, Dumbleton JH, Yuan HA**.** Biomaterials. 1996 Jun; 17(12): 1157-67.

- **Spine arthroplasty: a historical review:** Szpalski M, Gunzburg R, Mayer M. Eur Spine J. 2002 Oct; 11 Suppl 2: S65-84. Epub 2002 Aug 13.

- **Artificial intervertebral discs and beyond: a North American Spine Society Annual Meeting symposium.** Blumenthal SL, Ohnmeiss DD, Guyer R, Hochschuler S, McAfee P, Garcia R, Salib R, Yuan H, Lee C, Bertagnoli R, Bryan V, Winter R**.** Spine J. 2002 Nov-Dec; 2(6): 460-3

SKIN REPAIR:

- **Skin tissue engineering,** Bannasch H, Fohn M, Unterberg T, Bach AD, Weyand B, Stark GB., Clin Plast Surg. 2003 Oct; 30(4): 573-9.

- **Update on new biomaterials and their use in reconstructive surgery,** Curr Opin Otolaryngol Head Neck Surg. 2003 Aug; 11(4): 240-4. **Pou AM.**

- **The use of fibrin glue in skin grafts and tissue-engineered skin replacements: A review,** Currie LJ, Sharpe JR, Martin R**.,** Plast Reconstr Surg. 2001 Nov; 108(6): 1713-26.

- **Tissue-engineered skin. Current status in wound healing,** Bello YM, Falabella AF, Eaglstein WH., Am J Clin Dermatol. 2001; 2(5): 305-13.