IMPLANTABLE DEVICES: AN OVERVIEW

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INTRODUCTION

Textile fibres, yarns, fabrics, composites and 3-D shaped fabrics from woven, knitted, nonwoven, braided and embroidery processes play a vital role in the manufacture of various implants, including the replacement of diseased or non-functioning blood vessels and segments of the aorta or other big arteries. It is even feasible to produce vascular prostheses as fine as 2 to 3mm in diameter(1). This overview briefly discusses the importance of various textile materials and products used for implanting in the human body for periods ranging from few weeks to permanent implants for replacement of diseased or non-functioning parts.

Table 1 summarises the main types of textile structures used for various healthcare and medical devices, including implants.

Table 1 Fabric Structures Used in Various Healthcare and Medical Devices

Woven	Gauze dressings, compression bandages, plasters, vascular prostheses, scaffolds, surgical gowns, drapes and hospital textiles such as sheets, blankets, pillowcases, uniforms and operating room textiles, implants, knee supports and braces.
Nonwoven	Surgical gowns, caps and masks, absorbent layers, wipes, fleeces, protective clothing, diapers, feminine hygiene products, incontinence products, wound dressings, scaffolds, implants and antidecubitus fleece.
Knitted	Compression bandages, vascular prostheses, stents, heart valves, ligaments and tendons, surgical hosiery, compression hosiery, compression hosiery, blankets, wound dressings, stockings, elasticated net garments, pressure garments, finger bandages, support bandages, flat bandages and spacer materials for knee braces, implants, nets and hammocks.
Crochet	Compression bandages for compression therapy, cast cloth for orthopaedic casting bandages, wound dressings, bandages and implants.
Embroidery	Implants.
Braiding	Sutures, soft tissue ligaments and implants.
Composite Materials	Diapers, feminine hygiene, incontinence products, wound dressings, scaffolds, implants and support systems for treatment of pressure ulcers.

VASCULAR PROSTHESIS

The first artificial vascular graft was produced from polyamide fibre in 1956. Polytetraflouroethylene (PTFE) fibre soon replaced polyamide and then polyester fibre was introduced(2). The implants are made from a variety of synthetic materials. The main fibres include polyester, PTFE, polypropylene and polyacrylonitrile. However, polyester and PTFE are the most common vascular prostheses currently available.

The major requirements of a good vascular graft include:

- biocompatibility;
- non-fraying;
- flexibility;
- durability;
- stability to sterilisation;
- resistance to bacteria/virus; and
- nonthrombogenicity.

Knitted polyester vascular prosthesis has become the standard vascular graft for replacement of arterial vessels of 6mm and greater. However, while this has many features required by a surgeon, such as ease of handling, saturability and conformability, it has one major disadvantage: it is not blood-tight. The knitted structure, by its nature, is porous, which is what is required for rapid incorporation by tissue ingrowth from the host. At the time of surgery the surgeon has to "pre-clot" the graft using some of the patient's own blood, which is taken before heparinisation – a time consuming process which can be difficult to carry out satisfactorily. This prevents its use when patients are heparinised such as for cardiopulmonary bypass and in emergency aneurysmal surgery when preclotting is not possible.

ADVANTAGES OF GELATIN IMPREGNATED GRAFT

Preclotting of grafts before implantation incurs additional operative time and patient blood loss as well as the risk of airborne infection and viral infection associated with blood transfusion.

The other major disadvantage of polyester fibre is its initial high thrombogenicity by luminal deposition of radio-labelled platelets, particularly in the period prior to graft maturation. Pre-sealing of knitted Dacron by protein impregnation with either collagen or gelatine renders the porosity of the material to zero, and such grafts are implantation-ready without pre-clotting. The gelatine sealant is chemically similar to that of gelatine-based plasma expanders, which have been safely used for many years, and the sealant demonstrates a number of important features in addition to its primary function of sealing the graft:

Table 2 Porosity of Vascular Graft Before and After Impregnation

Graft	Type	Porosity (ml $mm^{-1}cm^{-2}$)
Micron*	Warp knitted Dacron (1)	1900
Oschner*	Woven Dacron	500
Hemaguard-K*	Collagen impregnated (1)	0
Protegraft DV**	Warp knitted Dacron (2)	1900
Unigraft**	Gelatin impregnated (2)	0

* Intervascular
** B Brauen Medical

- Gelatin impregnation (Gelsoft – trademark of Vascutek Limited) hydrolyses over a period of 14 days by a non-enzymatic mechanism that does not elicit a prolonged inflammatory response.
- Gelatin has been shown to have low thrombogenicity. Under test conditions, gelatine-impregnated Dacron attracted fewer platelets than unimpregnated Dacron.
- The gelatine impregnation degrades over a period of 14 days, allowing for unimpaired tissue incorporation into the interstices of the knitted fabric skeleton of the Gelsoft graft.
- The Gelsoft double-velour base fabric is designed with a low internal velour pile height of 30 microns and an external velour pile height of 75-100 microns. These characteristics aid the production and maintenance of a thin, stable, firmly attached pseudo-intima and further encourage the incorporation of the graft into the surrounding tissue.

In recent research carried out at Cardiac Research Unit, Killingbeck Hospital, Leeds, by a group of surgeons, it was established that collagen and gelatine impregnation of Dacron vascular grafts eliminates the need for pre-clotting and also appears to reduce early thrombogenicity in vitro. Table 3 compares prostheses in controlled experiments. Figure 1 illustrates the effect of pre-sealing Dacron vascular prosthesis on mean graft thrombogencity index (GTI)(3).

Table 3 Mean Graft Thrombogenicity Index [GTI] (95% CL)

$$GTI = \frac{Mean\ graft\ radioactivity\ per\ cm}{Mean\ blood\ radioactivity\ per\ ml\ *}$$

* [blood radioactivity at (T_0 minute + T_{60} minutes) ÷ 2]

Micron	1.13	(0.87 – 1.38)
Oschner	1.12	(0.71 – 1.53)
Hemaguard-K	0.45	(0.40 – 0.51) pre-sealed
Protegraft DV (2)	1.95	(1.30 – 2.60)
Unigraft DV	0.06	(0.02 – 0.09) pre-sealed

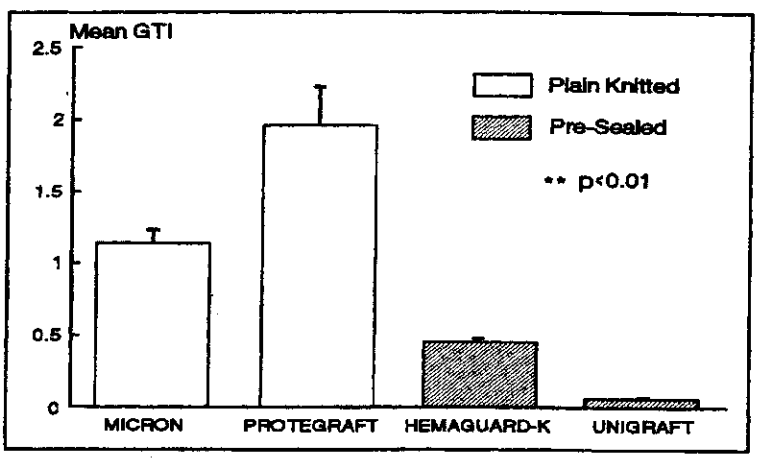

Figure 1 Mean graft thrombogenicity index (GTI)

LIGAMENT PROSTHESES

Carbon fibres, having high strength, stiffness and low creep properties are employed to replace damaged ligaments (connecting two or more bones) and tendons (connecting muscles to bones).

The knee is stabilised by four ligaments, namely:

- the anterior cruciate ligament (ACL);
- the medial collateral ligament (MCL);
- the lateral collateral ligament (LCL); and
- the posterior collateral ligament (PCL).

ACL is a very important stabiliser of the femur on the tibia and serves to prevent the tibia from rotating and sliding forward during jumping, running and other quick or sudden physical movements. However, the ACL can also be easily torn, and is the ligament which is frequently injured. Polyester and carbon fibres are frequently used in the manufacture of ACL prostheses. Proflex ligament is made of PET polyester fibre and consists of multiple braided tubes(4).

Gore-Tex is a knitted cable with eyelets on each end for fixation(5). Surgicraft UK, has carried out extensive research in the design, development and evaluation of ACL prosthesis. Their design is based on a number of strands, each of which is constructed from polyester and carbon fibre yarns, braided together with all 24 strands braided again together with eyelets formed at each end of the prosthesis. It has been established through extensive testing that the stress-strain characteristics of this prosthesis is very similar to a young human ACL. The combined properties of polyester and carbon fibres braided strands impart desirable properties, such as high strength, high elongation at break, high specific energy, low creep, high shear modulus and correct stiffness.

MESH GRAFTS

Body organs can be repaired using mesh grafts. The grafts are made from a fabric of a net which contains an open texture and evenly spaced holes. The utilisation of mesh grafts in humans is based on the fact that, during the absorption period, a neomembrane is formed in the site where the mesh has been implanted(6).

Mesh grafts are also used to aid skin healing. Clinical trials have been conducted to replace conventional "human cadaver allegraft skin" (HCAS) with a polyamide mesh fabric for the treatment of burn wounds.

One substitute for HCAS is Dermagraft-TC, developed for severely burned patients by the US company Advanced Tissue Sciences. Dermagraft-TC consists of human neonatal fibroblasts cultured on a Biobrane polyamide mesh fabric which is covered with a thin layer of silicon membrane as an epidermal barrier(7).

Some applications of mesh grafts on human beings include:

- treating a damaged kidney by external splinting or encapsulation;
- repairing a damaged pelvic peritoneum;
- replacing membranes covering the brain; and
- repairing abdominal wall defects (hernia).

RESORBABLE POLYMERS

Resorbable polymers are degraded in the body by hydrolytic or enzymatic cleavage of polymer chains and metabolism to simple molecules, which in turn are eliminated(8).

Polymers commonly used for medical implants are primarily polyester of alpha-hydroxycarbonic acids polyglycolic acid (PGA) and polylactide (PLA). PLA can be synthesised from L-lactide or D-lactide, resulting in a crystalline polymer. Copolymers or PLA synthesised from racemic D, L-lactide, are, however, amorphous and differ in their thermal, physical and degredation properties from crystalline polymers(8).

RESORBABLE SPUNLAID NONWOVENS

Resorbable spunlaid nonwovens are used increasingly as a substrate for three-dimensional tissue engineering, such as chondrocytes, endocrine cells, and as patches to cover larger areas of injured tissue. Nonwovens have a high porosity which allows permeation and growth of cells in vitro and in vivo.

As part of a BRITE EURAM research project, spunlaid and meltblown nonwovens were produced from a number of different resorbable polymers at ITV, Denkendorf, Germany, to study their tensile and other relevant properties for use as scaffolds for tissue engineering.

A number of applications of these materials for replacement of parts of non-functional tissues and organs were investigated and discussed during this research project; as below(9):

a) scaffolds for in vitro biohybridisation of cartilage;
b) biohybrid liver assist device; and
c) tracheal prostheses.

Figure 2 illustrates the sequence of implantation of cartilage at the ear. Autologeous cartilage cells are harvested from the patient and grown in culture, attaching to the fibres and producing collagen and other matrix proteins. The scaffold provides only a temporary support and is resorbed when new cartilage is formed. The process can be applied for hyaline (joint) or fibrous (ear) cartilage(9).

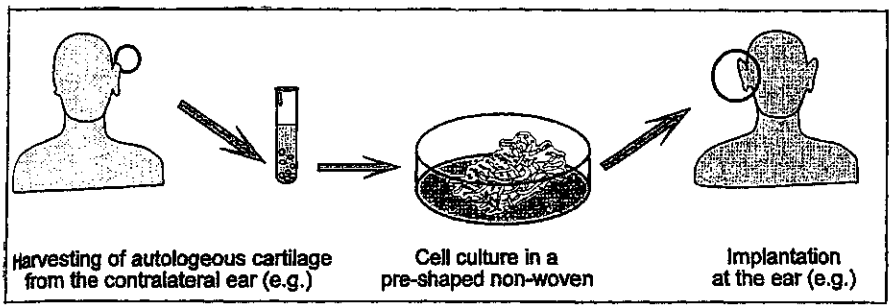

Figure 2 Manufacturing of an biohybrid auricle implant

For a more comprehensive review of implants and meshes, the reader is referred to the textile progress monograph recently published by the Textile Institute(10).

REFERENCES

1 M Yeabsley, Karl Mayer Textile Machinery Co Ltd; UK, Private Communication, April 2005.

2 J Moreland, *Medical Textiles*, August 11 1997.

3 S C Anand, 'Knitting's contribution to developments in medical textiles', *Textile Technology International,* 1994 220.

4 P S Freudiger, *Technical Textiles International,* 1994 **5** 12.

5 F R Noyes, D J Butler, E S Grood, R F Zemicke and M S Hefzy, *J of Bone Joint Surgery,* 1984 66-A 344.

6 S Rajendran and S C Anand, 'Developments in medical textiles', *Textile Progress,* 2002 **32**(4) 24.

7 S Rajendran and S C Anand, 'Applications of textile materials and products in healthcare', *Technical Textile Markets 2nd Quarter,* 2001 42.

8 M Dauner and H Planck, 'Biomedical materials for surgical implants', *Textile Asia,* February 1999 33.

9 M Dauner, 'Textile scaffolds for biohybrid organs', *Textile Asia,* October 1998 47.

10 S Rajendran and S C Anand, 'Developments in Medical Textiles', *Textile progress,* 2002 **32**(4) 22.

REPAIR OF ARTICULAR CARTILAGE DEFECTS USING 3-DIMENSIONAL TISSUE ENGINEERING TEXTILE ARCHITECTURES

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ABSTRACT

The successful repair of articular cartilage must rely on the restoration of the full thickness of the layer with full structural integration laterally with the existing material on the joint surface. The knee is the most commonly affected joint for cartilage degeneration and it is this joint that will be used as the basis for our investigations. Previously, textile mesh structures (based on intermeshed yarns) have been designed and used to provide an oriented repair of biological tissue in the body e.g. the repair of hernia in men. The repair of articular cartilage defects in lesions of the knee has also been described using a nonwoven structure with random fibre orientation into which repair tissue grows to provide a load-bearing surface. It is proposed that shape data (using the selection criteria of cartilage thickness < 1 mm and associated underlying bony changes) is generated in 3D space by imaging methods, and used together with structural information from the current knowledge base arising from previous studies and published material. This will allow a computer model of the architecture to be constructed. The 'male' form of the shape data will be used to design and manufacture textile engineered implants. It is envisaged that the 'female' shape around the lesion could be used as a surgical positioning tool to cut out (If necessary) the desired shape of the lesion to seat the final implant material. It is anticipated that a textile implant of pre-defined structure will be in a rigid or semi-rigid form, with the 'zonal' information relating to the structural architecture of the lesion built-in. In any generic class of textile material, including nonwovens, there is no single form of 3D structural architecture and this can be manipulated during manufacture. This has been overlooked in other studies using textile scaffolds. The fibre orientation, the porosity and the physical properties of the structure may be adjusted within wide limits. Different generic textile structures containing yarns, mechanically entangled fibres, films and membranes can also be combined depending on the required functionality of the material. Using the shape and thickness data obtained from imaging, it is proposed to design and evaluate textile structures of selected physical and mechanical properties that would reproduce a repair of similar structural design to the original articular cartilage at that site.

INTRODUCTION

Articular cartilage has a well-defined structure in its intact full thickness form [Kirk, et al, 1993]]. The zonal architecture is well known and the orientation of the superficial fibres within the Benninghoff arcades [Benninghoff, 1925]] can be determined on any joint

surface by staining pinpricks with Indian ink [Meachim et al. 1974]. The successful repair of articular cartilage must rely on the restoration of the full thickness of the layer with full structural integration laterally with the existing material on the joint surface. The knee is the most commonly affected joint for cartilage degeneration and it is this joint that will be used as the basis for our investigations. It is envisaged that the generic technology developed for the knee would be transferable to other joints in the body. Previously, textile mesh structures (based on intermeshed yarns) have been designed and used to provide an oriented repair of biological tissue in the body. The repair of articular cartilage defects in lesions of the knee has also been described using a nonwoven structure with random fibre orientation into which repair tissue grows to provide a load-bearing surface [Minns et al,1989]. Fixation with the lateral tissue occurred with time and after 6 months or more the fibrocartilagenous material has differentiated into mature articular cartilage [Muckle and Minns,1990, Minns et al 1993, Minns et al, 1995].

At present the majority of patients offered total joint replacement are in their sixth decade onwards. Although described as "total" replacement, not all cases have the patella resurfaced. There is an increasing trend to replace only "half" the joint (Unicondylar Arthroplasty) in cases where only one compartment is involved. In the past these cases, which had an associated varus deformity, were managed initially with a high tibial osteotomy. The track record of current prostheses with 10-year survivals in the order of 95% makes their use in this age group acceptable.

However, there remains a large group of patients who present a difficult management problem. These are patients of a younger age group who do not respond to conservative treatment. Initial management would include simple analgesics and non-steroidal anti-inflammatory agents. The next stage would be to consider intra-articular steroid injection. A recent development is to use intra-articular hyaluraonic acid injection. Arthroscopic washout is a further option but this and injection therapy is only effective for the order of 6-12 months.

There is a further group of patients, often presenting in their 30's, suffering from isolated patello-femoral wear. An alternative less invasive procedure than total joint replacement would have an important place in the management of the above patients. Such a procedure would need to preserve as much bone stock as possible and have the ability to be tailored to the individual patient's cartilage lesion rather than replace an entire articular surface as in unicondylar replacement for example. It is important to consider why the particular lesion has arisen in the first place, and address any abnormal loading or instability of the joint. e.g. patellar mal-tracking.

Although articular cartilage substitutes have been developed for treatment of chondral and osteochondral defects, to date none has successfully replaced normal articular cartilage. In operative treatments two basic repair options are available: 1) Regeneration of the damaged tissue by new cells and matrix, and 2) Substitution or replacement of the damaged tissue with biological or synthetic materials that possess mechanical properties similar to those of articular cartilage [Jackson et al,2001]. Whereas previous attempts are cartilage repair has aimed to achieve either regeneration or substitution the approach outlined in this invention is distinctive because it provides elements of both. An implant that simulates the basic macrostructural elements of cartilage is described which is semi-rigid. This structure is designed to guide cell proliferation when *in situ* to assist in the process of self-repair and may also act as a delivery system if pre-seeded with cells prior to implantation. A nonwoven implant is provided in which the basic macrostructure is designed to simulate the

cross-sectional structure of healthy cartilage including the archadial elements[Minns and Steven, 1976].

Potential future revision of the new procedure to total knee replacement would not have to be compromised using this resurfacing concept.

MATERIALS AND METHODS

The distal femur of 10 mature pigs were cleared of adhering soft tissue and muscle and mounted using plaster-of-Paris within a cylinder of high density polyethylene (HDP) such that the whole distal surfaces of the femoral condyles were exposed. A flat plate made of HDP with a circular depression for positioning of the cylindrical mount was placed within the RF coil of the MRI machine. A wooden dowel of 3 mm diameter was pushed into a drill hole on the patella and on the patellar trochlea of the corresponding femur to hold the patella in position during the imaging.

To produce replica mouldings of the articular surfaces, a ring made of HDP with three locking screws was put over the mounting cylinder and locked such that the articular surface could be replicated when inverted. The replication mould was produced by ensuring the level of the moulding plaster covered the whole of the articular surface. Stone plaster was used for the replication moulding as this composed of sub micron sized powder which gave a very smooth surfaced mould and did not shrink during curing giving an accurate representation of the surface geometry.

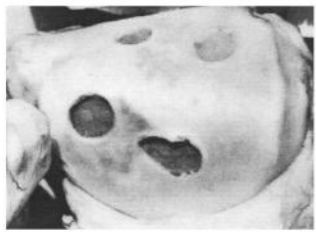
Osteochondral lesions of various shapes were reproduced by a high-speed flat-bottomed router of 3 mm diameter to a depth of 3 mm, on both the pig and Ox specimens. The minimum and maximum widths of these osteochondral lesions were recorded using Vernier scale callipers with a measuring accuracy of +/- 0.1 mm. These dimensions were recorded before imaging and replication mouldings taken to ascertain any geometrical shrinkage or distortion artefacts would occur.

A GE shielded magnet 1.5 Tesla SIGNA MRI machine was used which produced MRI fat-suppressed gradient echo sequence images of 1 mm thick slices in the sagittal and coronal plane (TR=14 ms, TE=6.8 ms, FA=20 degrees, bandwidth=15, FOV=14, 256x192 matrix, NEX=1) were taken over the whole articular surface of the distal femur or patellae. The articular cartilage thickness on the edge of the biopsy hole was measured from the images and correlated with the direct measurements. A computer generated 3D image of the shape was produced by non-contacting profiling methods used in rapid prototyping techniques of the replication moulds.

The MR image data was recorded in DICOM format on CD and converted into three-dimensional computer models using specialist software. The accuracy of these virtual models was then correlated to the casting data.

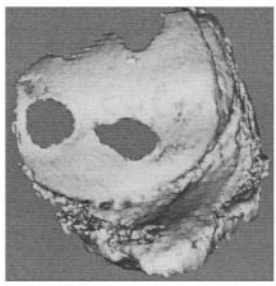
Physical models were then produced directly from the MR image data by the stereolithography process [Swaelens et al, 1996]. The stereolithography process constructs physical models directly from computer data in a layer-by-layer process. The stereolithography apparatus (SLA) creates models from a liquid resin that cures to solid when exposed to ultra violet light. The computer model is sliced into a number of thin layers. Then an ultra violet laser scans the first slice onto the surface of the resin with a laser to produce a solid layer. The build platform is lowered by one layer thickness and resin floods over the solidified layer. A recoating blade levels the resin and the next layer is scanned on top of the first, and so on until the model is complete [Bautsch et al, 1994].

MRI data is captured as a series of planar images with a gap between them typically in the order of 1 mm, whilst the stereolithography process builds models at a layer thickness of typically 0.15 mm. Therefore interpolation is used to create intermediate sections between the original images. A cubic interpolation ensures that the intermediate sections are anatomically accurate and natural. These sections then directly drive the rapid prototyping machine that builds the physical model . Thirdly, this two-and-half-dimensional format can be more accurate and less memory intensive than three-dimensional approximation formats such as the commonly used triangular faceted STL file. The views of a typical example of articular lesions induced on a pig patella, at the various stages of production are shown in figure 1, and the shape data can be used in conjunction with the production process described below to produce custom-made implants [Munjal, et al, 2000].

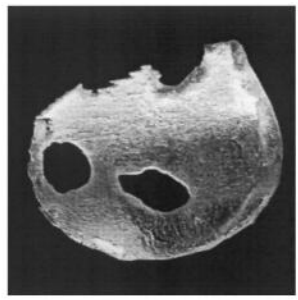


Ox patella lesions

MRI section of same



Reconstructed image



3d resin model

Fig. 1

DESIGN OF A SYNTHETIC 3D SCAFFOLD WITH AN ARCHADIAL MACROSTRUCTURE

Nonwoven materials are constructed from fibres (length to breadth ratio >1000:1) or continuous filaments based on resorbable or non-resorbable synthetic and natural polymers. The fibres or filaments are assembled in to a web structure whose geometry is characterised by packing density, pore size distribution and the x,y and z orientation of the fibre components. Initially, nonwoven webs tend to have a planar layered structure but they can

be made with a pronounced z-directional fibre orientation either during or immediately after web forming. The structural geometry of nonwoven materials is complex (see figure 2) and at first sight fibres can appear to be randomly oriented. This is partly because of the requirement for mechanical interlocking of fibres to stabilise the web and makes the formation of a web containing unidirectional fibres impractical. Autogeneous frictional bonding is essential to construct an initial web structure that can be further processed. Web geometry is then further manipulated in subsequent mechanical bonding steps where characteristic z-directional structures are formed by groups of fibres, which are induced to migrate from the original x,y planes in the web. Pronounced z-directional orientation can thus be produced.

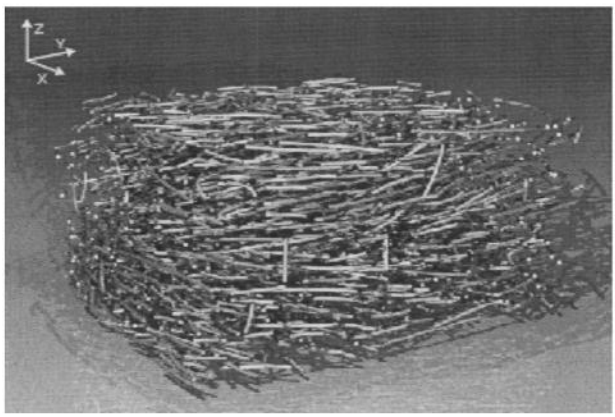


Fig. 2

The plan is to construct a conformable and porous 3D scaffold structure, with macrostructural features that are characteristic of cartilage. This scaffold must be permeable to cartilage cells and culture medium and therefore, the scaffold should not be a microstructural analogue of cartilage as the pore size would preclude cell migration in to the structure.

We will examine strategies for forming cell permeable archadial structures in the z-direction of fibrous nonwoven webs of up to 3-5mm thickness composed of resorbable fibres. These regions will be connected to a nonwoven surface in which a planar x,y fibre orientation dominates. Thus, the fabric structure will be heterogeneous in the cross-section and will have defined pore geometry. Pilot-facilities are available at Leeds for the preparation of such scaffolds and prototypes will be constructed using commercially available fibre and filament raw materials. The methods we will use to induce z-directional orientation will include migration of fibre groups using fluid forces (hydroentanglement), mechanical entanglement using barbed needles and combinations thereof. This will be combined with studies of stuffer-box web deformation approaches which generally have the effect of reorienting a large proportion of fibres in to the z-direction e.g. micrex-creping and STRUTO-slitting processes. It is envisaged that secondary bonding of these structures using hot-air convection bonding (through-air) of the thermoplastic components will be necessary to set the required architecture. The internal geometry of the fabrics will be

studied and quantified using ESEM, SEM and x-ray tomography with dedicated image analysis packages developed by the research group. Pore characteristics will be quantified using porometry.

CONCLUSIONS

A semi-rigid implant is proposed containing textile fibres of resorbable or non-resorbable material arranged in a three-dimensional structure and suitable for use as an implant in the repair of articular cartilage defects or lesions. The shape data can be very accurately obtained by non-invasive means utilizing MRI imaging that delineates articular cartilage lesions. An implant that simulates the basic macrostructural elements of cartilage is described which is semi-rigid and is the shape and geometry of the imaged lesion to produce a custom-made implant that would fit precisely at surgery. This structure is designed to guide cell proliferation when *in situ*, to assist in the process of self-repair and may also act as a delivery system if pre-seeded with cells prior to implantation. A nonwoven implant is provided in which the basic macrostructure is designed to simulate the cross-sectional structure of healthy cartilage including the archadial elements.

REFERENCES

T L Bautsch, E E Johnson and L L Seeger, 'True three-dimensional stereographic display of 3D reconstructed CT scans of the pelvis and acetabulum', *Clin Orthop*,1994 **305** 138.

A Benninghoff, 'Form und bau der gelenkknorpel in ihren Beziehungen zu Funktion', *Zeitschaft fur Zell und M Anatomie*, 1925 **2** 783-862.

D W Jackson, M J Scheer, T Simon, 'Cartilage Substitutes: Overview of Basic Science and Treatment Options', *J of the American Academy of Orthopaedic Surgeons*, 2001 **9**(1) 37-55.

T B Kirk, T B, Wilson, A S and G W Stachowiak, 'The morphology and composition of the super ficial zone of mammalian articular cartilage' *J Orthopaedic Rheumatology*, 1993 **6** 21-28.

G Meachim, D Denham, I H Emery, and P H Wilkinson, 'Collagen alignments and artificial splits at the surface of human articular cartilage', *J Anat*, 1974 **118** 101-118.

H Migaud, B Corbet, C Assaker, J F Kulik and A Duquennoy, 'A. Value of a synthetic osseous model obtained by stereo-lithography for preoperative planning. Correction of a complex femoral deformity caused by fibrous dysplasia', *Rev Chir Orthop Reparatice Appar Mot*, 1997 **8**(2) 156.

R J Minns and F S Steven, 'The collagen fibril organisation in human articular cartilage', *J.Anat*,1976 **123** 437-457.

R J Minns and D S Muckle, 'Mechanical and histological response of carbon fibre pads implanted in the rabbit patella', *Biomaterials*, 1989 **10** 273-276.

R J Minns, D S Muckle, and J A Betts, 'Biological resurfacing using carbon fibre', *International Orthopaedics*, 1993 **1** 414-424.

R J Minns, T K O'Brien, P England, J A Betts and D S Muckle, 'Biological resurfacing of the knee-a review of surgical management', *The Knee*, 1995 **1** 197-200.

D S Muckle and R J Minns, 'Biological response to woven carbon fibre pads in the knee' *J Bone and Joint Surg*, 1990 **71B** 60-62.

S Munjal, S S Leopold, D Kornreich, S Shott and H A Finn, 'CT-generated 3-dimensional models for complex acetabular reconstruction', *J Arthroplasty*, 2000 **15** 644.

B Swaelens, Noorman van der Dussen and Vancraen, *Medical Models Using Rapid Prototyping and Manufacturing Techniques,* Stereolithography and other RP&M Technologies (Ed Paul F Jacobs) Pub ASME Press, New York, 1996, 339-347.

MANUFACTURE AND *IN VITRO* BIOACTIVITY OF SOL-GEL-DERIVED SILICA FIBRE AND P(L/D,L)LA COMPOSITE

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Keywords: Sol-Gel, Silica Fibre PLA Composite, SBF, Calcium Phosphate Formation, Si release

ABSTRACT

Silica fibres and nonwoven fibre assemblies were prepared from TEOS-derived silica sols using sol-gel method and dry spinning technique. The same sols can be used to prepare different fibre structures and to control the biodegradation. Due to the glassy nature with a low elasticity and a porous 2-5 nm structure, the observed fibres were alone difficult to process by using mechanical methods. In order to avoid that, the nonwoven fibre was further hot-pressed onto one side of the melt-processed poly(L/DL)lactide 70/30 (PLA70) membrane. The aim was to study the *in vitro* biodegradation (silica release) and bioactivity of the fibre composites. The *in vitro* bioactivity and dissolution of the fibres were studied in a simulated body fluid. To monitor the surface topography and roughness of the silica fibres, a scanning probe microscopy with a tapping mode atomic force microscopy (AFM) was used. It was shown that it is possible to prepare silica fibre composites, which are able to form calcium phosphate *in vitro*. The calcium phosphate formation ability could be further guided to one side of the composite membrane with the help of pure silica fibres.

INTRODUCTION

In general, fibres have been used to improve mechanical properties of materials. They have also been used in biomaterials applications, where their use, however, is not limited only to the improvement of mechanical properties. Fibres having active surface properties or suitable porous structure can be used as such or as a bioactive part in composites. Bioactive and biodegradable silica fibres, which have been studied only to a minor extent [1-4], provide alternatives for the design of novel products, for example, membranes used in tissue guiding and bone repairs or biodegradable carrier materials for biologically active agents. *In vitro* bioactivity (here: calcium phosphate formation ability in SBF) of sol-gel-derived silica fibres have been studied in our previous papers [2-4]. The higher the viscosity in the spinning sol, the better the bioactivity. Furthermore, the bioactivity was improved for every sample, as they were heat-treated at 175°C. The results indicated that the surface topography in the nanometer scale is the most important factor controlling the *in vitro* bioactivity of the mildly heat-treated fibres. In addition, the dissolution of the fibres could be varied in a wide range without having a significant effect on the bioactivity [2-4]. This is an important fact, because

recent studies have shown the importance of the dissolving silicon on the osteoblast proliferation and differentiation [5,6]. These fibres are too brittle for many load-bearing medical applications. Mechanical properties can be improved with intensive heat-treatment, but it is obvious that biodegradation and bioactivity is reduced due to denser structures and decreased surface reactivity, respectively. The problems with mechanical properties and mouldability can be overcome by preparing composites with polymers. In this study silica fibres were hot-pressed onto a melt-processed PLA membrane. The *in vitro* properties of the sol-gel derived silica fibre-PLA composites have not been studied earlier. The aim of this experiment was to study the *in vitro* biodegradation (silica release) and bioactivity of the fibre composites.

Methods

The silica fibres were prepared using the sol-gel method and dry spinning technique. The preparation process of the fibres has previously been described in detail [1-4]. The silica sols were prepared from tetraethylortosilicate (TEOS), deionized water, absolute ethanol, and HNO_3 as catalyst. Typical molar reaction composition of sol consist of 2:1 water:TEOS and 1:1 ethanol:TEOS. The share of acidic hydrolysis catalyst (HNO_3) varied from 0,037 to 1, and was in case of partly neutralized catalyst (HNO_3 : NH3OH) of 0,10:0,01, as presented in Table 1.

TABLE I
Sol Compositions in Molar Ratios and the Viscosity of the Sol Before Spinning the Fibres

Sample Name	R=H_2O/ TEOS	EtOH/ TEOS	HNO_3/ TEOS	NH_3/ TEOS	Viscosity (Pas)
FIB (A)	2	1	0.036	0	2.0*
FIB (A)	2	1	0.1	0	2.0*
FIB 3	2	1	0.1	0.01	2.0†
FIB 1 (B)	2	1	0.036	0	3.9†
FIB 2 (B)	2	1	0.1	0	3.0†
FIB 1 (C)	2	1	0.036	0	15*

*The viscosity of the sol before spinning the fibres at 0°C;
†the viscosity of the sol before spinning the fibres at 20°C.

The gels obtained showed a very narrow spinning window between the low viscosity fluid with a rather high surface tension and the rapid increase of viscosity up to gelation, as presented in Table 2. Reaction time before spinning was strongly affected by acid concentration and was drastically reduced by using partly neutralized media. It was observed, that the gelation process was partly continuing after fibre formation, and thus a heat treatment at 175 °C was carried out. A scanning-transmission electron microscopy was used to study the bulk structure of the green state fibres, and the main chemical composition of fibres was analysed by using FT-IR spectrometric analysis. The biodegradability of fibres was measured by using the salt-buffered simulated body fluid (SBF) containing 6 % of tris(hydroxymethyl)-aminomethane.

Table 2

Spinnability times for the sols prepared at 40°C

Sample name	Reaction time until spinnable (*h*)	Spinnable at RT (*h*)
FIB1 (A and B)	400–450	>8
FIB2 (A and B)	210–215	5–7
FIB3	68–69	3–4

The fibres were bonded together to form a nonwoven assembly by a direct spun-laid technique. The dried nonwoven was further heat-treated at 175°C (10°C/h from 25°C to 175°C → 4 h at 175°C → slow cooling to 25°C). The heat-treated nonwoven was hot-pressed (125°C) onto one side of the melt-processed poly(L/DL)lactide 70/30 (PLA70) membrane. The thickness of the membrane was 0.3 mm. The total fibre content of the composite was about 20 wt-%. As control samples we used pure PLA and continuous fibres as such, which we prepared and heat-treated in the same way as the nonwoven assemblies. The *in vitro* bioactivity of the composite and dissolution of the active fibres were studied in SBF [7] at pH 7.40 and 37°C (10 mg fibres in 50 ml SBF, also the composite was normalized to contain 10 mg fibers in 50 ml SBF). Sample solutions were monitored for calcium and silicon concentrations as a function of the immersion time using atomic absorption spectroscopy (AAS) and UV-Vis spectroscopy, respectively. The composition and morphology of the samples, after 14 d immersion in SBF, were analyzed with a scanning electron microscopy coupled with an energy-dispersive X-ray analyser (SEM-EDX). The surface topography and roughness of the silica fibres before immersion was determined by the non-contact tapping mode atomic force microscopy (AFM) using a NanoScope III multimode AFM (Digital Instruments, Santa Barbara, CA) apparatus.

Results

Fibre properties

The best continuous fibres are achieved with samples FIB1B and FIB2B, however the filament easily breaks and further mechanical processing is difficult, even if the heat treatment was carried out or the fibre was aged for a longer period. The transmission electron microscopic studies showed a quite smooth fibre surface and a porous structure of 2-5 nm, which is typical for sol-gel processes applying various phases, as seen in Fig. 1.

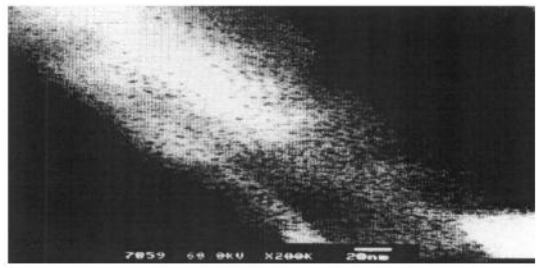

Figure 1. A transmission electron microcraph for the green body of FIB2 B aged for 3 months.

The FT-IR measurements made for the heat-treated fibres are shown in Fig. 2. They give further contribution for the suggestions after TGA losses at 300 oC of some organic material present within the silica structure. Thus, peaks at 2230 cm^{-1} and 3050 cm^{-1} are clearly seen in FIB1A and FIB2A, but they can not be directly related to any component present in the system. The only possibility is, that the fibres contain carbon residuals, which may form double bonds with oxygen observed at these points.

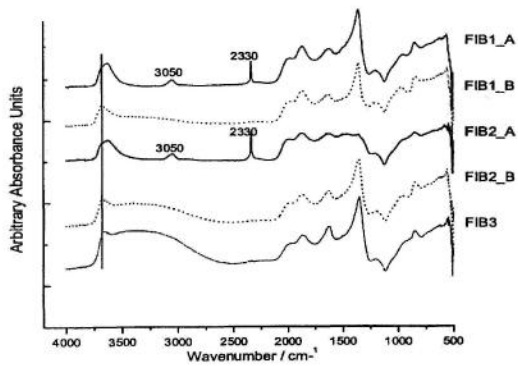

Figure 2. FT-IR spectra for the fibres samples heat treated in the thermogravimetric analysis.

The thermal treatment affected differently the fibres manufactured by different recipes, as shown in Fig. 3. The spinnable fibres can be devided into 3 groups estimated from the starting spinning viscosity: $\eta < 3.0$ Pas ; $\eta = 3.0 - 5.0$ Pas ; $\eta = 15.0$ Pas, presented according to the decreased weight change in Fig. 3, respectively.

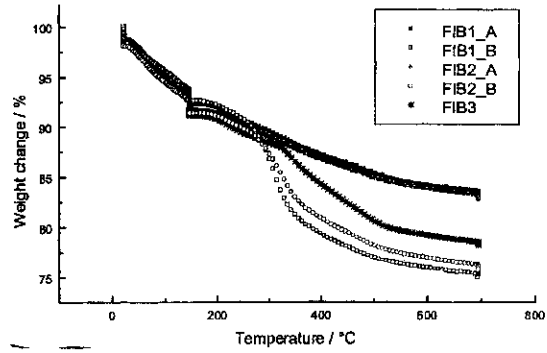

Figure 3 . Thermogravimetric spectra for the green state fibre samples aged for 3 months.

First biodegradability tests in the SBF of the green state fibres, showed that some kind of plateau or a saturation level is achieved after a few days of immersion. The solubility rates of FIB1 B, FIB2 B and FIB3 are clearly faster than for FIB1 A and FIB2 A. This indicates that in the structure of the fibres spun in the later stage of spinning window, the area of silica available for the degradation has to be greater. The biodegradation data of certain samples are illustrated in Fig. 4.

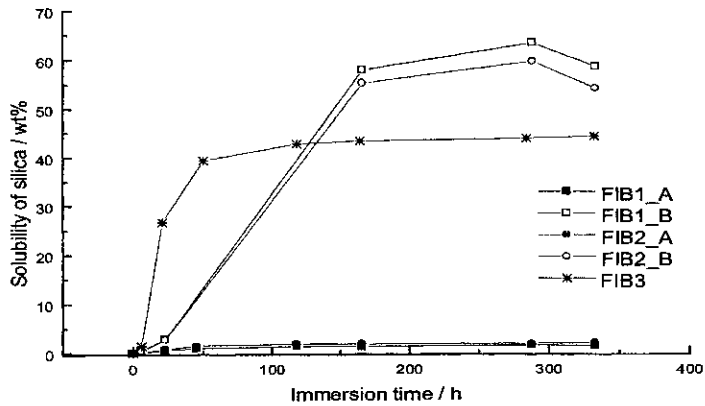

Figure 4. Biodegradation of the green fibre samples aged for 1 month.

The behaviour of fibre composites

The decrease of calcium concentration in SBF indicates the calcium phosphate formation on sample surfaces. The *in vitro* bioactivity results are shown in Fig. 5a and in SEM images in Fig. 6. As shown in Fig. 5a the calcium phosphate (CaP) formation started within 1 day for the control fibres heat-treated at 175°C and within 4 days for the

corresponding dried fibres (50°C). The CaP nucleation and growth scene was similar for the nonwoven fibre and composite. The CaP formation started within 4 to 7 days. As shown in Fig. 5a there was no CaP deposition on the pure PLA surface. For the composite the CaP deposition was observed only on the one side of the membrane. Furthermore, CaP deposits can be seen clearly only on the silica surfaces, but not so much on the adjacent PLA surfaces.

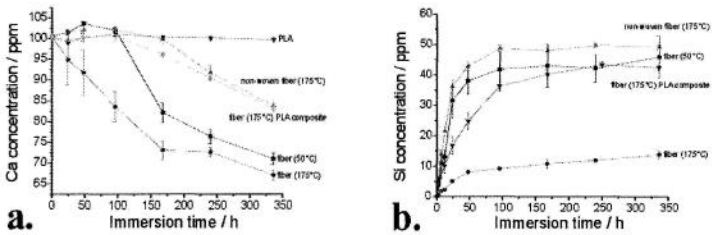

Fig. 5: a) The change of calcium concentration in SBF as a function of immersion time for the fibres and fibre composite b) The Si solubility of the silica fibre samples in SBF as a function of the immersion time.

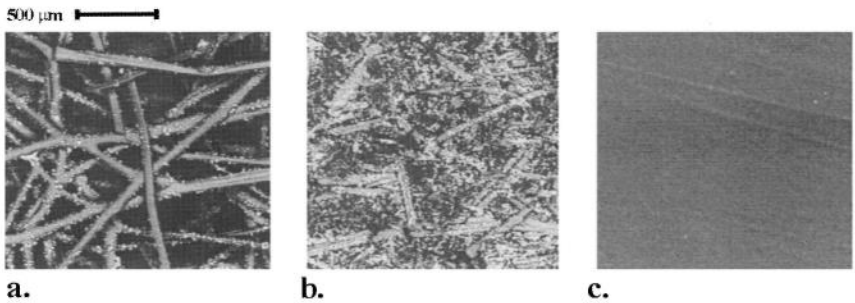

Fig. 6: The surface of a) nonwoven fibre, b) fibre-PLA composite, and c) PLA after 14 days immersion in SBF. Note lighter calcium phosphate precipitation on a and b surfaces.

As shown in Fig. 5b the constant Si level (50 ppm) and the dissolution rate (1.8 ppm/h: calculated from the linear portion of the curves) of the nonwoven fibre was higher compared to the fibre composite (40 ppm and 0.8 ppm/h). The lowest Si solubility was obtained for the control fibres (10 ppm and 0.2 ppm/h). The corresponding dried fibres were much more soluble (40 ppm and 1.1 ppm/h).

The line profiles for the silica fibre samples, obtained from the AFM section analysis, describing the vertical surface topography are shown in Fig. 7. These line profiles represent the average image of studied surfaces obtained from 9 separate section analyses. For the control fibre obtaining the best *in vitro* bioactivity, the peak heights are greater and the surface is rougher compared to other samples. For the nonwoven fibre

and fibre PLA composite obtaining similar CaP formation ability, the surface roughnesses are quite similar.

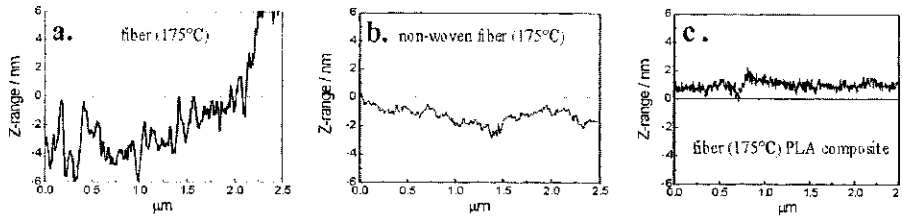

Fig 7. The line profiles for the fibre samples obtained from the AFM section analysis.

DISCUSSION AND CONCLUSIONS

It was shown that the rougher the silica surface in the nanometer scale, the better the bioactivity. This finding is in accordance with our previous studies showing similar bioactivity for the similar surface roughness [3]. However, the surface properties and the reactivity are quite different between the control fibres and nonwoven fibre assembly. These samples were prepared using same precursor ratios at same conditions, but from different sol batches pointing out the sensitivity of the process. The preparation of the nonwoven assembly, using the direct spun-laid technique, should not have an influence because there is no extra treatment included in the process. As shown earlier, a careful control of the sol, spinning parameters, ageing and heat-treatment are needed to prepare homogeneous fibres [2]. Further, it was shown that the CaP formation ability of the pure nonwoven fibre assembly was retained in the fibre-PLA composite, i.e., we were able to transfer fibres onto the PLA surface without affecting their *in vitro* bioactivity. The CaP formation ability can be further guided to one side of the composite membrane with help of these active silica fibres.

The low Si solubility of the control fibre is suggested to be due to the thick CaP layer (not shown) reducing the Si to release. The differences in the Si solubility between composite and fibres are due to the differences between "free" silica surfaces meaning that the diffusion path is longer and more difficult in composites. In recent studies the importance of dissolving silicon on the osteoblast proliferation and differentiation has been shown [5-6]. It is suggested that relatively high steady state silicon concentration (10–20 ppm) in cell culture (or in tissue) induce osteoblast proliferation and differentiation [6]. For the studied sol-gel-derived silica fibres in addition to the reactive and bioactive surfaces it is also possible to adjust the silicon release near to the osteoblast activating level. As mentioned earlier the dissolution of the fibres could be varied in a wide range without having an effect on the bioactivity [1-4]. For example, in this study the constant Si level reached is between 10 to 50 ppm and dissolution rates are 0.2-1.8 ppm/h for bioactive fibres and fibre composites. These dissolution rates may locally result in suitable levels if the removal of Si is slow, as it can be assumed to be in bone tissue. Low-temperature processing also provides an additional benefit; it is possible to add drugs into the system during the fibre preparation [8]. As shown in this study the low-temperature processed fibres (50°C) also obtained quite good ability to form CaP and some drugs also tolerate moderately elevated temperatures used in our processes.

In summary, it was shown that it is possible to prepare *in vitro* bioactive silica fibre-PLA composites. The CaP formation ability can be further guided to one side of the composite membrane with the help of the active silica fibres on the PLA surface. This kind of bioactive silica fibre-PLA composite membrane with optimal bioactivity and the adjustable Si release is very beneficial with respect to the tissue guiding and bone repair applications.

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REFERENCES

1 M Jokinen, T Peltola, S Veittola, H Rahiala and J B Rosenholm, *J Eur Ceram Soc*, 2000 **20/11** 1739.

2 T Peltola, M. Jokinen, S Veittola, H Rahiala and A Yli-Urpo, *Biomaterials,* 2001 **22/6** 589.

3 T Peltola, M Jokinen, S Veittola, H Rahiala, J Simola and A Yli-Urpo, *J Biomed Mat Res,* 2001 **54/4** 579.

4 M Jokinen, T Peltola, S Veittola, J Simola and A Yli-Urpo, *Bioceramics* **14,** *Key Engineering Materials* 2002 **218-220** 283.

5 I Xynos, A Edgar, L Buttery, L Hench and J Polak, *J Biomed Mater Res,* 2001 **55** 151.

6 L Hench, *Bioceramics* **13** *Key Engineering Materials* 2001 **192-195** 575.

7 T Kokubo, H Kushitani, S Sakka, T Kitsugi, T Yamamuro, *J Biomed Mater Res,* 1990 **24** 721.

8 T Czuryszkiewics, J Ahvenlammi, P Kortesuo, M Ahola, M Jokinen, F Kleitze, M Lindén and J B Rosenholm, *J Non Cryst Sol* 2002 **306(1)** 1.

A SPIDER SILK SUPPORTIVE MATRIX USED FOR CARTILAGE REGENERATION

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ABSTRACT

Injured cartilage, not accreting by itself, often decreases the quality of life. The chondrocytes need an implanted support to bridge and recover the wound with extra-cellular matrix products forming fresh cartilage. Advances in cell biology and biomaterial research have lead to new possibilities in tissue engineering. Transplanted scaffolds, holding a 3D cell culture, should copy the cartilage characteristics. Strength and flexibility are important, but even more an adequate porosity, so the chondrocytes can migrate through the matrix, but are not able to float around.

Looking for regeneration and not a repair, we want the scaffold material to disappear while real cartilage is healing the wound. In this way the material and its hydrolysis products have to be biocompatible and harmless. In the case of synthetic polymers, the hydrolysis products are frequently toxic, Spider silk is a promising fibre for many applications. Completely made out of protein a suspected biocompatibility is already proven. The harmless amino acid hydrolysis products make the silk a good candidate for creating a bioresorbable textile scaffold.

The chondrocytes cells adhere quite well on the spider cocoon silk threads. Cocoons can be obtained each autumn in large numbers from the Araneus diadematus garden spider. The mechanical properties of the silk are more appropriate than polymeric gels, like hyaluronic acid, collagen and alginate, which have proved to be successful in 3D immobilisation and maintaining the differentiated phenotype of chondrocytes. The phenotypical products collagen II and aggrecan were also detected around the cells growing on the spider cocoon silk. A silk 3D textile could possibly be applied in combination with a polymer gel, probably alginate, in order to achieve some biomechanical stability. While biodegradation is occurring, the silk textile is overgrown with real cartilage and eventually the wound will be recovered without any definitive synthetic implants.

SILK IN BIOMATERIALS

Silk from the silkworm, *Bombyx mori*, has been used as surgical suture for centuries (Altman et al, 2003). But the interest of material and textile engineers in spider silk has increased during the last few decades. The extraordinary combination of strength and flexibility makes the spider silk fibre competitive with high-performance synthetic fibres like Kevlar (Kubic, 2002). Due to the predatory nature of spiders and the low level of the production, these threads have not been commercialised yet.

Advances in biotechnology have made it possible to reproduce spider silk-like proteins in mammalian cells (goats) (Lazaris et al, 2002) and in plant-cells (tomato and tobacco) (Scheller et al, 2001). The goal is now to spin the extracted spider silk-like proteins into

fibres with the same mechanical properties as their natural counterpart. This promises a lot of futuristic applications, which will be the first to be utilised in the world of medicine. The strength of these fibres makes them valuable to replace certain tissues like cruciate ligaments. (Altman et al, 2002) The proteinic nature makes it more biocompatible than synthetic materials. Some biological responses to silkworm silk material has raised questions about hypersensitivity, but it has already been proven that this would be due to the sericin glue-like proteins around the silk core. Virgin silk, when the sericin is removed by boiling in Na_2CO_3 aqueous solution, seems to be biocompatible and can be a good substrate for cell-growth (Minoura et al, 1995). Spider silk is not coated with any sericin proteins. Nevertheless, animal experiments are going on in our laboratories to examine the immunological reactions towards spider silk fibres.

Proteins are polymers that are composed of amino acids, which are harmless and can be assimilated in the human body after degradation of the proteinic fibre, like lactic and glycolic acid when PGLA are used. This is useful in regenerative medicine, one of the most progressive areas of tissue engineering. In regenerative medicine tissues are replaced just temporarily by a degenerative material forming a scaffold for tissue cells which will overgrow the biodegrading scaffold and form a new living tissue (Solchaga et al, 2001)

The extraordinary combination of the strength and elasticity of the silk fibres are due to a mixture of crystalline beta-sheets and amorphous helical and coiling regions. (Gosline et al, 1986). Almost the entire sequence of the silk proteins consists of repetitive elements, which makes it unlikely that a random structure can be generated. Poly-Ala and Poly-Gly-Ala are forming 2 different crystalline beta-sheets. Alanine can produce a larger hydrophobic interaction than Glycine, which makes the Poly-Ala-betasheets stronger, than the Poly-Gly-Ala-betasheets. Other repetitive elements like GGX and GPGXX are responsible for helical and beta-spiral conformations (Hayashi et al, 1999). A widely accepted hypothesis is that the secondary structure consists of three parts. A crystalline phase made of large beta-sheets making extra-molecular interactions and an amorphous phase in which the third phase composed of small crystals is lying. (Takahashi et al, 1991)

Unlike silkworm silk, spiders can produce more than one fibre, several silk genes have already been discovered (Gatesy et al, 2001) and there is a silk gland for each of the 7 different silks found. All these silk fibres have a different amino acid sequence leading to a different molecular structure correlated to their function. Draglines should be stronger than the capture threads in the spider web and this suits with the abundant poly-Ala-regions in dragline compared to those in other spider silk threads like the capture threads where the elasticity is more important than the strength. All this is the result of million years of evolution and an example for material engineers to make fibres or protein scaffolds that can imitate the mechanical properties of the tissue that is replaced.

NONWOVENS IN BIOMATERIALS

Usage of fibres in biomaterials differs from other polymer material. Synthetic polymers can be polymerised or melted in a specified shape and by using salt-leaching an adequate porosity can be obtained. When natural fibres like silk are used, or other fibres are processed, the right textile-technique has to be used to fulfil the requirements for a good scaffold material (Ko et al, 1997). A scaffold should have a certain shape and volume so it can replace the tissue or fill a wound. More important is the porosity. Cells should be able to grow, multiply and migrate through the scaffold. The porosity should also be

homogenously divided over the scaffold. The fibres should not be spun into yarns and woven into a textile fabric. The porosity in a yarn is too small for a cell and the space between the yarns is too large. A nonwoven textile is the best solution to make a fibrous scaffold for tissue regeneration (Doser et al, 2000). Techniques like spunlacing and needlepunching can be used to fix the fibres and provide the fabric an adequate and homogenously distributed porosity (watzl et al, 2000).

CARTILAGE REGENERATION

Cartilage wounds do not heal fully by themselves and cause a lot of pain and impair the function. No decent cure has been found until now, although a lot of research has been done on cartilage repair (Hunziker, 1999, 2002). Such lesions are generated during the course of many joint diseases; osteoarthritis, in conjunction with genetic or metabolic conditions, such as acromegaly, Paget's disease, the Stickler-syndrome and hemophilia or as a result of trauma. Diseased tissue is now removed by shaving, debridement or laser abrasion. In order to heal the lesion, autologous tissue transplantation showed some neoformation of cartilage-like tissue during in vivo studies, but clinical experience with human patients has never been successful.

The hope is set on tissue engineering, structurally and functionally reconstituting mammalian tissue. For cartilage repair chondrocytes isolated from a small piece of cartilage from the patient, smaller than needed for autologous tissue transplantation, can be cultured in vitro. Or embryonic or mesemchymal stem cells can be cultured and differentiated to chondroblasts and chondrocytes with the use of the correct growth- and differentiation factors, like TGF-β or by inducing the mechanical stimuli like real cartilage. These cells should grow in a scaffold that has the same properties as the extra-cellular matrix of real cartilage, strong enough, but not too strong so the chondrocytes are still triggered to express the right genes so they produce the characteristic cartilage products like aggrecan and collagen II.

Already existing matrices can be divided in four classes; protein-based, carbohydrate-based, artificial and combined polymers. The collagen matrices are the best known of the protein-based ones. Out of the Carbohydrate-based polymers chondrocytes are phenotypically more stable on alginate substrates than on PL/PGA or agarose. To improve the cell-adhesion sometimes RGD-sequences are attached on the polymers (Coutts et al, 2001). Textile matrices made out of spider silk fibres or new materials based on spidroins could be a new form of protein-based material for cartilage scaffolds.

CHONDROCYTE GROWTH ON SPIDER SILK

Human chondrocytes were isolated and grown on spider cocoon silk to examine the adhesion, the growth and the phenotypic stability of the cartilage cells.

Materials and methods

Araneus diadematus, better known as garden spiders, were grown in the lab. Cocoons are obtained at the end of their lifecycle. The cocoons were sterilized by autoclavage. Human chondrocytes were isolated out of cartilage from the knee. The cells were put on the cocoon fibres and the fibres were transferred to an agarose layer or embedded in alginate

after 24 hours so only the cells attached on the fibres were maintained. In a MEM-medium with bovine serum added, refreshed 3 times a week, the cells were grown for 1, 2 and 3 weeks. After these periods paraffin embedded samples were examined by immunohistochemistry with monoclonal antibodies against aggrecan and collagen type I and type II.

Results and discussion

The chondrocytes cells adhere quite well on the spider cocoon silk threads. The chondrocytes-products collagen II and aggrecan were always found in accreting amount. Collagen I, was only found after 3 weeks in a small quantity. This means the phenotype of the cells remained during the first weeks without the use of any growth factors. Alginate has already proven to create a good substrate for cartilage cell-growth; it is inert for the cell's phenotype. But the hydrogel, which can create a preshaped form, isn't solid enough to hold the pressure and shear in joints. A combination with spider silk can solve this problem. In this experiment the alginate embedding did not change the cell growth and ECM-production much. A silk 3D textile structure could be applied possibly in combination with a polymer gel, alginate or a silk regenerated fibroin gel in order to achieve some biomechanical stability. While biodegradation is occurring slowly, the silk textile is overgrown with real cartilage and eventually the wound should be recovered without any definitive synthetic implants.

Native spider silk cocoon threads were used as a substrate for chondrocytes. The spider silk fibres supported the growth of the cells and the production of an ECM The maintaining of the phenotypic stability over a 3 week period proves that spider silk can be a good basic material for scaffolds for cartilage regeneration. This research was carried out with cartilage material and native silk threads but an extension to other soft tissues and synthetically made spider silk-like fibres should be investigated.

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REFERENCES

G H Altman, F Diaz, C Jakuba, T Calabro, R L Horan, J Chen, H Lu, J Richmond and D L Kaplan, 'Silk-based biomaterials', *Biomaterials*, 2003 **24**(3) 401-16.

G H Altman, R L Horan, H H Lu, J Moreau, I Martin, J C Richmond and D L Kaplan, 'Silk matrix for tissue engineered anterior cruciate ligaments', *Biomaterials*, 2002 **23**(20) 4131-41.

R D Coutts, R M Healey, R Ostrander, R L Sah, R Goomer and D Amiel, 'Matrices for cartilage repair', *Clin Orthop*, 2001 (391 Suppl) S271-9. Review.

M Doser and H Planck, 'Tissue engineering – new possibilities of tissue and organ regeneration', *Nonwovens industrial textiles*, 2000 (3) 10.

J Gatesy, C Hayashi, D Motriuk, J Woods and R Lewis, 'Extreme diversity, conservation, and convergence of spider silk fibroin sequences', *Science*, 2001 **291**(5513) 2603-5.

J M Gosline, M E DeMont and M W Denny, 'The structure and properties of spider silk', *Endeavour*, 1986 **10**(1) 37- 43.

C Y Hayashi, N H Shipley and R V Lewis, 'Hypotheses that correlate the sequence, structure, and mechanical properties of spider silk proteins', *Int J Biol Macromol*, 1999 **24**(2-3) 271-5.

E B Hunziker, 'Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects', *Osteoarthritis Cartilage*, 2002 **10**(6) 432-63. Review

E B Hunziker, 'Articular cartilage repair: are the intrinsic biological constraints undermining this process insuperable?', *Osteoarthritis Cartilage*, 1999 **7**(1) 15-28. Review

F K Ko, 'Textile structures for surgical implants', *Textile Asia*, June 1997.

S Kubik, 'High-performance fibers from spider silk', *Angew Chem Int Ed Engl*, 2002 **41**(15) 2721-3.

A Lazaris, S Arcidiacono, Y Huang, J F Zhou, F Duguay, N Chretien, E A Welsh, J W Soares and C N Karatzas, 'Spider silk fibers spun from soluble recombinant silk produced in mammalian cells', *Science*, 2002 **295**(5554) 472-6.

N Minoura, S Aiba, Y Gotoh, M Tsukada and Y Imai, 'Attachment and growth of cultured fibroblast cells on silk protein matrices', *J Biomed Mater Res*, 1995 **29**(10) 1215-21.

J Scheller, K H Guhrs, F Grosse and U Conrad, 'Production of spider silk proteins in tobacco and potato', *Nat Biotechnol*, 2001 **19**(6) 573-7.

L A Solchaga, V M Goldberg and A I Caplan, 'Cartilage regeneration using principles of tissue engineering', *Clin Orthop*, 2001 (391 suppl) S161-70.

Y Takahashi, M Gehoh and K Yuzuriha, 'Crystal Structure of Silk (Bombyx mori)', *J of Polymer science*, 1991 **29** 889-891.

A Watzl and J Eisenacher, 'Spunlaced and airlaid nonwovens for medical textiles', *Technical textiles*, 2000 **43** 295-298

NONWOVEN SCAFFOLDS OF IMPROVED DESIGN FOR THE TISSUE ENGINEERING OF THE ANTERIOR CRUCIATE LIGAMENT

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ABSTRACT

This work is concerned with improving nonwoven scaffold design for the tissue engineering of the anterior cruciate ligament. When designing a scaffold two important design criteria to consider are scaffold's internal structure and biocompatibility, both of which are addressed in this paper.

The role of a scaffold is to provide a framework for cells to attach, proliferate and secrete extra cellular matrix. The scaffold also acts as a template, directing the growth of cells and newly formed tissue. It is the scaffold's internal structure, together with polymer surface chemistry and morphology, which directly influence the cellular activities that lead to tissue formation.

With regard to scaffold's internal structure, structural parameters are discussed in relation to specific scaffold function; for example the effect of scaffold pore-size on cell proliferation, migration and nutrient supply. Another structural factor discussed is the role of fibre orientation as a means of guiding and organising new tissue growth.

With the aim of creating a scaffold of optimum design, for the tissue engineering of the anterior cruciate ligament, nonwoven scaffolds of differing structure have been constructed. In order to understand the relationship between manufacturing method and scaffold structure characterisation techniques have been employed to analyse the structural parameters of these scaffolds. Obtained scaffold structural properties are discussed in relation to manufacturing method.

Regarding the second scaffold criteria biocompatibility tests have been conducted by the authors on a range of generic fibre types. The results of these tests are provided in the form of cell attachment, with reference to fibre morphology.

INTRODUCTION

Tissue engineering merges the fields of cell biology, engineering, materials science and surgery to fabricate new functional tissue using living cells and a matrix or scaffold [1]. In order to engineer a neo-tissue (or neo-organ) the following are required; a scaffold (either temporary or permanent) on which to generate the neo-tissue, appropriate cells or migrating host tissue from which to form the neo-tissue, culture medium for nutrient supply and waste removal and finally the optimum culturing conditions. These optimum culturing conditions can be either within the body (*in vivo*) or outside the body (*in vitro*), mimicking *in vivo* conditions.

Concerning the scaffold, it is the internal structure of the scaffold that helps determine the type of neo-tissue generated. Scaffold structural parameters control the specific cellular activities, leading to tissue formation. By altering these structural parameters it is possible

to optimise the generation of tissues such as neo-ligaments, e.g. the anterior cruciate ligament (ACL).

In addition to the scaffold's structure, cell-substrate biocompatibility also influences the cellular activities leading to neo-tissue formation. If the scaffold substrate is not found to be biocompatible cell attachment will be poor and subsequent tissue generation may be inadequate. Substrate biocompatibility can be assessed through seeding the substrate with a known quantity of cells and monitoring for cell necrosis or proliferation.

STRUCTURAL DESIGN PARAMETERS OF SCAFFOLDS

Tissue engineering scaffolds are three-dimensional structures that assist in the tissue engineering process by providing a site for cells to attach, proliferate, differentiate and secrete extra-cellular matrix, eventually leading to tissue formation. These scaffolds can be either permanent or temporary in nature, depending on the application and the function of the neo-tissue. Temporary scaffolds are made from biodegradable polymers, such as polyglycolic acid, which degrade within the body to leave a purely biological neo-tissue [2]. Permanent scaffolds remain within the body, working with ingrown tissue to form a polymeric/biological composite [3].

For neo-tissue formation to occur cells must first be able to penetrate the scaffold sufficiently; giving way to a high cell seeding density. This requires that the scaffold be highly porous, with a high surface area to volume ratio. As most cells are anchorage dependant and require attachment to a solid surface for viability and growth [4], a high surface area is essential for high cell growth rates [5]. Research has shown that a scaffold porosity of at least 90% is ideal, for specific scaffold-cell interactions, nutrient and waste diffusion and sufficient space for ECM regeneration within the scaffold [6].

Other structural parameters governing cell penetration and migration within the scaffold are pore-size [5] and pore interconnectivity. If the pore-size is too small cells will be unable to penetrate the scaffold and migrate within it, producing uniform cell seeding throughout. If the pore-size is too large cells will be unable to bridge the pore during cell proliferation: inhibiting effective neo-tissue generation.

It is possible to guide cells, and newly formed collagen fibres, into the desired configuration though orientating the scaffold substrate accordingly. Research has found that when random and parallel oriented scaffolds were seeded with cells, collagen fibre orientation was enhanced within the parallel oriented scaffold in the early stages of static, *in vivo,* culturing [7].

NONWOVEN SCAFFOLD MANUFACTURE

In this study nonwoven manufacturing methods were chosen to produce scaffolds to be later used in the tissue engineering of the anterior cruciate ligament. The large surface area to volume ratio and, in general, the greater opportunities for manipulating the structure and mechanical properties of the scaffold, were considered to be advantageous over other scaffold manufacturing methods, such as gas foaming [8].

Nonwoven scaffolds used to compare partially engineered tendons include polyglycolic acid (PGA) scaffolds of random and oriented structure [7]. With the longitudinally oriented scaffolds consisting of unbraided PGA fibres, knotted at one end, and the random scaffolds being nonwoven PGA mesh, many structural dissimilarities exist between the two

structures. This makes it difficult to compare tissue formation within these structures and directly attribute a particular scaffold structural parameter to neo-tissue formation.

In order to create a scaffold of optimum design for the tissue engineering of the anterior cruciate ligament it is first necessary to understand how the manufacturing method influences the structural parameters of the scaffold. In this study those structural parameters known to influence the cellular activities leading to tissue formation are characterised in random and oriented nonwoven scaffolds. One such structural parameter is fibre orientation distribution. Various methods have been established to measure fibre orientation in nonwoven fabrics [9], however for reasons beyond the scope of this paper it was necessary to create a new method using Image Pro® Plus software.

Method

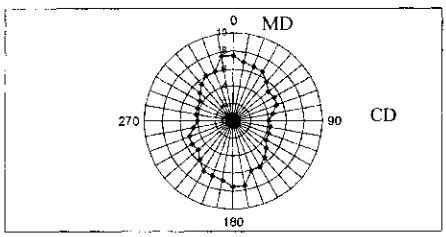
Staple polyester fibre (1.6dtex, 38mm, Dupont, USA) was used in the production of longitudinally oriented batts through the parallel-laying of carded webs. Conversely, random batts were produced by composite-laying of carded webs, at pre-determined angles. Both parallel-laid and composite-laid batts were needle punched at needle penetration depths of 8mm, 10mm and 12mm. In the construction of both scaffold structures approximate batt weights of 110 g/m^2 were used.

Scaffold mass per unit area and thickness were measured following standard test procedures BS EN 29073-1 and BS EN ISO 9073-2, respectively. Scaffold pore-size distribution was measured (n=4) using a Capillary Flow Porometer (Porous Materials Inc., USA), following standard test procedure ASTM F316.

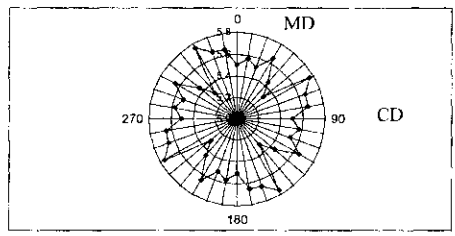
Optical microscopy (x 17mag.) and Image Pro® Plus software (Media Cybernetics®, USA) were used to develop a macro to measure fibre orientation on the scaffold surface (n=4). Using this macro it was possible to determine the orientation of fibre segments, typically 1500, relative to the machine direction (0°).

Results

It was found that scaffold thickness decreased with increasing needle penetration depth and fabric packing density increased with increasing needle penetration depth, in all manufactured scaffolds. With increasing fabric packing density the fabric porosity decreased for all scaffolds.



1: Polar plot for 8mm needle-punched parallel-laid scaffold.

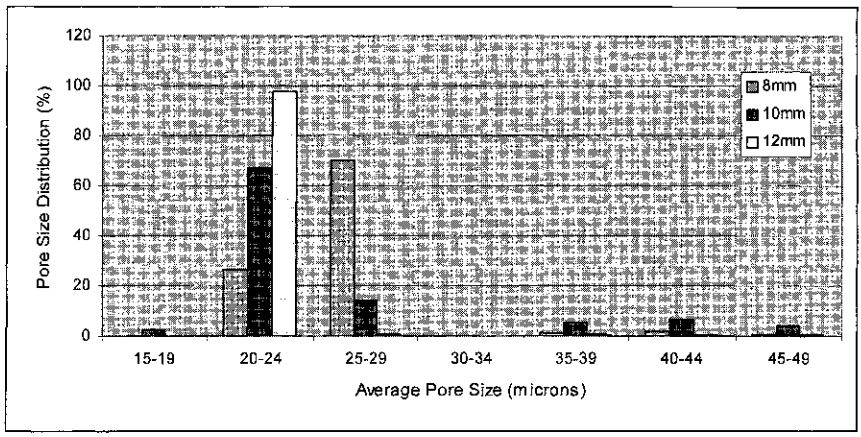


2: Theorised polar plot for 8mm needle-punched composite-laid scaffold.

A polar plot created from the mean fibre orientation on the surface of the 8mm needle-punched parallel-laid scaffold, illustrated a predominant fibre orientation in the machine

direction, see figure 1. The polar plot of the 8mm needle-punched composite-laid scaffold, known to be of random orientation, can only be theorised using this macro. Measuring fibre orientation on the surface of the scaffold and taking into consideration the angles between the web layers and the total number of webs used to achieve the specified batt weight, a polar plot illustrating isotropic fibre orientation can be formed, see figure 2. Results of this study suggested no obvious trend for calculated MD/CD (fibre orientation) ratios of the parallel-laid scaffolds, at increasing needle penetration depths.

Concerning the pore size distribution in the parallel-laid and composite-laid scaffolds, a trend was observed with respect to modal pore size distribution, generally ranging from 20 to 30 μm. It was found that the percentage of the modal pore size distribution increased with increasing needle penetration depth. This is illustrated in figure 3 for the needle-punched composite-laid scaffolds.



3: Pore size distribution for needle punched composite-laid scaffolds

Discussion of Results

Scaffold's structural parameters related to the scaffold macrostructure include mass per unit area, thickness and packing density. Results of this study found that an increase in needle penetration depth, in parallel-laid and composite-laid scaffolds, led to a decrease in scaffold thickness and an increase in packing density. This was to be expected, with increasing needle penetration depth leading to an increase in fabric consolidation.

During this study another scaffold macrostructural parameter, fibre orientation, was altered using batt forming methods to produce anisotropic (parallel-laid) and isotropic (composite-laid) scaffolds. This change in scaffold anisotropy was illustrated using polar plots, with preferential fibre orientation in the machine direction for parallel-laid scaffolds and a theorised randomised fibre orientation for composite-laid scaffolds. Theorised polar plots for composite-laid scaffolds, together with inconclusive calculated MD/CD (fibre orientation) ratios for parallel-laid scaffolds highlight the need for further work in the determination of three-dimensional fibre orientation in scaffolds; this may include the use of x-ray micro-tomography, which is currently being investigated.

Scaffold microstructure can be used to gain a better understanding into those cellular activities leading to tissue formation, within the scaffold. One of the main structural parameters responsible for influencing cell penetration into and migration throughout the scaffold is pore size distribution. In this study it was observed that modal pore size distribution was generally in the range of 20 to 30 μm, for parallel-laid and composite-laid scaffolds. Research has found an optimum pore size of 20 μm for fibroblast cells [10], indicating that fibroblast cells would be able to penetrate a major volume of the scaffolds tested. It was also observed that the percentage of the modal pore size distribution increased with increasing needle penetration depth. Three possible mechanisms, resulting from needle-punching, that could effect pore size are: drafting (causing an increase pore size), consolidation (causing a decrease in pore size) and fibre entanglement (causing a decrease in pore size). One or more of these mechanisms could have been the cause of the above; this area requires further work.

BIOCOMPATIBILITY TESTING OF GENERIC FIBRE TYPES

Cells attach to substrates via cell-surface receptors, which interact with proteins adsorbed onto the surface of the substrate [11]. These proteins are adsorbed from either the surrounding serum (culture medium or biological fluid) or secreted by the cells themselves [4]. With cells interfacing with these proteins, adhesive proteins are said to act as bridging molecules between the cells and substrate [12].

Research has found that low to moderately hydrophilic polymers support a high fraction of fibroblast cells [13]. In agreement with these findings it has been observed that cell adhesion [via adsorbed protein] appears to be maximised on those surfaces with intermediate wettability [4]. Although the hydrophobic polymers PTFE, PP, and PET have been noted to support limited cell attachment [13], other hydrophobic polymers such as polystyrene have demonstrated low cell attachment. It is thought that cell attachment to non-polar polymers, such as polystyrene, differs and maybe due to the crystallinity of the polymer, with poor cell adhesion being associated with amorphous materials [13].

Other substrate properties thought to enhance cell adhesion are positively charged surfaces [4] and grooved surface topographies, which maybe due to an increase in surface area [14].

Biocompatibility has been defined as the ability of a material, prosthesis, artificial organ, or biomedical device to perform with an appropriate host response in a specific application [15]. In the case of tissue engineering scaffold's biocompatibility is a function of the material from which the scaffold is constructed. Two properties that influence cell-substrate compatibility are substrate surface chemistry and surface topography. Based on this, present experimental research was designed to assess cell-fibre compatibility on a range of generic fibres. Biocompatibility tests were conducted to study the reaction of mouse fibroblast cells (L929) to a range of generic fibre types, viable cell attachment results were quantified 24 and 72 hours after cell seeding.

Materials

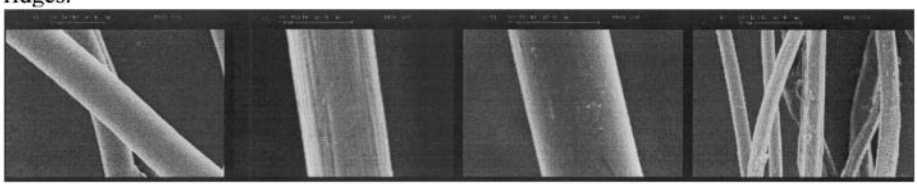
Eight polymers were evaluated in this study [16]: staple fibre polypropylene (PP) (Drake, UK), polyester (PET) (Dupont, USA), viscose rayon (Acordis, Germany), para-aramid (industry sourced), carbon (Soficar, France), polytetrafluroethylene (PTFE) (Lenzing,

Austria), poly-L-lactic acid (PLLA) (Cellon, Luxembourg) and polyglycolic acid (PGA) (Cellon, Luxembourg).

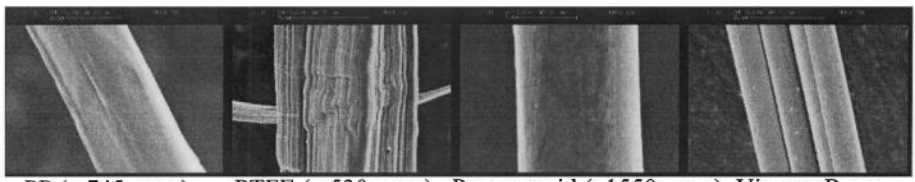
Scanning Electron Microscopy (SEM)

Since surface topography is one of the parameters that are thought to affect cell adhesion, fibres were evaluated by SEM. The images are illustrated in figure 4.

The surface topography of the PGA, para-aramid, PET and PLLA fibres were smooth. PP fibre was found to have shallow, irregular grooves on its surface and the carbon fibre to have slightly more regular, pronounced shallow grooves. The viscose rayon had wide striations along its length, which is a characteristic of the fibre due to wet spinning. The most irregular fibre surface was associated with the PTFE fibre, which possessed deep ridges.



PGA (x345mag.) Carbon (x1572 mag.) PET (x 1014 mag.) PLLA (x 168 mag)



PP (x 745 mag.) PTFE (x 530 mag.) Para-aramid (x1550 mag.) Viscose Rayon (x 1070 mag.)

4: SEM images illustrating surface topography of generic fibres used in biocompatibility experiment.

Experimental Method

Fibre Scouring and Disinfecting

Prior to conducting the biocompatibility tests any fibre finish that may have been present was removed using a filter sterilised (0.2µm) non-ionic synthetic detergent (Croscour, Eurodye-ctc, UK), which had been diluted using sterile water (Miza Pharmaceuticals, UK). Fibres were immersed in sterile 70% (v/v) ethanol solution for one hour to disinfect the fibres prior to testing. All procedures were performed using aseptic conditions.

Testing Apparatus & Cell Seeding

Scoured and disinfected groups of fibres, of pre-determined total surface area, were placed into plastic microbiological culture wells for testing. Washed (7x, ICN Biomedicals, Inc., USA) and sterilised stainless steel gauze (Expamet, UK) was used to position and retain the

fibres beneath the cell suspension level to ensure effective cell seeding. Aseptic techniques were used throughout.

Mouse fibroblast cells (L929, passage 15) were suspended in 3ml Dulbecco's Modified Eagles Medium (DMEM) (Sigma-Aldrich Co., Ltd., UK), supplemented with 10% (v/v) Foetal Calf Serum (Gibco Brl, Life Technologies Ltd., New Zealand), L-Glutamine (Sigma-Aldrich Co., Ltd., UK) and Penicillin and Streptomycin (Sigma-Aldrich Co., Ltd., UK). Cell suspension (3ml), containing 4.93 x 10^6 cells/ml, was seeded over the fibres at a cell seeding density of 2 x 10^4 cells/cm^2.

Cells seeded into tissue culture treated wells acted as a positive control. Other controls used included sample controls (fibres without cells) and a background control (medium only). Samples and controls were gassed with 5% CO^2 in air and incubated at 37°C for 1 day (n=4) and 3 days (n=4), after which time ATP-LITE™ (Perkin Elmer™ Lifesciences, USA) assays were conducted.

ATP

Assessment of cell attachment to fibres was conducted using the ATP-LITE™ assay measuring the level of ATP activity as an indication of viable cell attachment to fibres.

Cell seeded fibres were removed from culture wells and the fibres gently rinsed using Dulbecco's Phosphate Buffered Saline (PBS) (Sigma-Aldrich Co., Ltd., UK) to remove any non-adherent cells. Washed fibres were transferred to clean culture wells for cell lysing: 1ml of fresh supplemented DMEM culture medium was added to each well, followed by 0.5ml of Mammalian Cell Lysis Buffer (Perkin Elmer™ Lifesciences, USA). Wells were agitated for 5 minutes. Lysed cell solution was transferred to a 96-well Optiplate (150µl/well), to which a further 50µl/well ATP Cell Substrate Solution (Perkin Elmer™ Lifesciences, USA) was added and agitated for 5 minutes.

For the background control 2ml of the cell medium was removed from the well and 0.5ml of the Mammalian Cell Lysis Buffer was added. The remainder of the experimental method was followed. For the positive control cell suspension was removed from the wells and the wells rinsed with PBS, to remove any non-adherent cells. 1ml of fresh supplemented DMEM culture medium was added and the remainder of the experimental method followed. Assessment of viable cell attachment using the ATP-LITE™ assay was conducted.

Two way analysis of variance (two way ANOVA) and calculation of the minimum significant difference was used to determine a statistically significant increase in cell attachment relative to that occurring on the viscose rayon fibre following equivalent incubation periods.

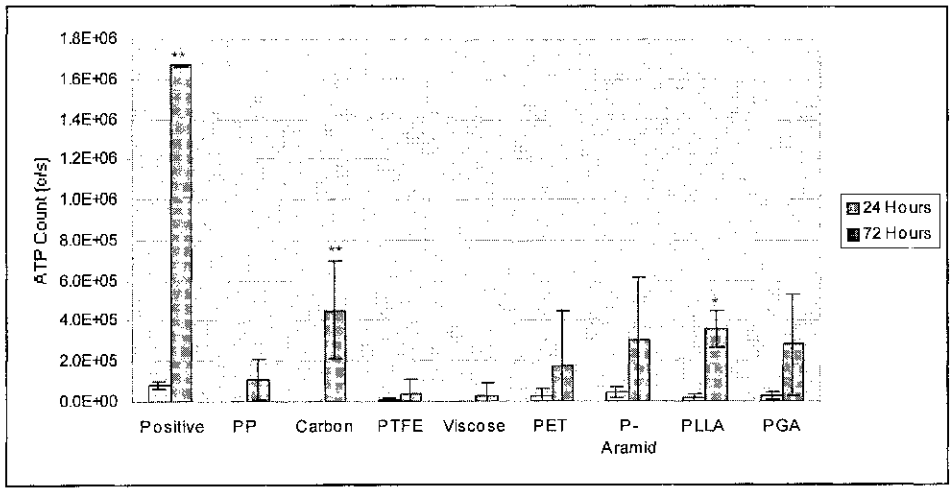
Results

The results of *in vitro* fibroblast cell attachment to a range of generic fibre types, differing in fibre properties, are illustrated in figure 5.

(24 Hours) Post Cell-Seeding

It was found that PP displayed no cell attachment 24 hours after cell seeding. Compared to the PP, a general trend could be observed in which cell attachment numbers increased with

the following fibres respectively: viscose rayon, carbon, PTFE, PLLA and PGA, with polyester and the para-aramid fibres displaying the greatest levels of cell. However this did not reach statistical significance.



5: Graph illustrating fibroblast cell attachment to a range of generic fibre types both 24 hours and 72 hours after cell seeding (± 95% confidence limits). Results have been adjusted for surface area. (* = 0.05, ** = 0.01, significantly greater cell attachment compared to viscose rayon)

(72 Hours) Post Cell-Seeding

The relative quantities of cells attached to the generic fibre types had altered considerably after 72 hours in culture. Viscose rayon had the lowest number of attached cells. Increasing levels of cell attachment were found for the PTFE, PP, PET, PGA, para-aramid and PLLA respectively, with the greatest level of cell attachment observed for the carbon fibre. It should be noted that the number of viable cells attached to the fibres 72 hours after cell seeding, was greater than after 24 hours, due to cell proliferation. Statistical analysis has revealed there to be significantly higher numbers of attached cells to the carbon fibre ($p=0.05$) and the PLLA ($p=0.01$), compared to the fibre with lowest cell attachment at 72 hours (viscose rayon).

These observed results support preliminary biocompatibility tests carried out by the authors and are supported by research in which fewer fibroblast cells were found to attach to PET fibres, when compared to para-aramid 72 hours after cell seeding [17].

Conclusion and Discussion

In the early stages of cell attachment, cell-substrate interactions are likely to be a function of the substrate surface chemistry [17], with optimum cell attachment to surfaces with low to moderate levels of hydrophilicity [13]. This was found to be the case for these experimental results, 24 hours after cell seeding, with the most hydrophobic polymers

supporting the lowest levels of cell attachment. With increasing hydrophilicity cell attachment was seen to increase, this was true for all fibres tested except for the viscose rayon, which may have been too hydrophilic. Research has found some highly hydrophilic polymers to support low levels of cell attachment [13]. Differences in initial cell attachment to the hydrophobic fibres may be accounted for by their relative surface topographies, with PP having shallow grooves and PTFE having deep ridges (which may have increased the relative surface area for cell attachment).

Subsequent cell attachment and proliferation 72 hours after cell seeding may be due less to the wettability of the substrate and more due to its topographic nature. Contrary to cited literature observation [14] it appears that cell numbers at 72 hours were higher on the smoother fibres (e.g. Carbon) as oppose to grooved fibres (e.g. PTFE). In order to fully understand these results other properties of the fibres used need to be characterised and taken into consideration in further experiments.

SUMMARY

In this paper the relationship between some of the cellular activities, which lead to tissue regeneration, and scaffold's structural parameters have been discussed. Leading on from this the structural characteristics of manufactured nonwoven anisotropic and isotropic scaffolds have been discussed in relation to manufacturing method. Finally the biocompatibility of scaffold substrates has been investigated, testing a range of generic fibre types for viable cell adhesion at 24 hours and 72 hours post cell seeding. This paper can be summarised by the following points:

- Tissue engineering scaffolds are three-dimensional structures that assist in the tissue engineering process by providing a site for cells to attach, proliferate, differentiate and secrete extra-cellular matrix, eventually leading to neo-tissue formation.
- For a scaffold to function effectively it must possess the optimum structural parameters, conducive to the cellular activities leading to neo-tissue formation; these include cell penetration and migration into the scaffold, cell attachment onto the scaffold substrate, cell spreading and proliferation and cell orientation.
- In this study it was found that manufactured parallel-laid and composite-laid scaffolds decreased in thickness and increased in packing density with increasing needle penetration depth. Modal pore size distribution was found to be in the range 20 to 30μm for all manufactured scaffolds: indicating that cell penetration and migration would occur within a majority of the scaffold structure.
- Biocompatibility tests conducted found no significant difference in viable cell attachment between tested fibres, 24 hours after cell seeding. However, a general trend was observed, with increasing cell attachment on increasingly hydrophilic fibres.

  A Significant difference in cell attachment was observed, 72 hours after cell seeding, between the fibre with the least cell attachment (viscose rayon) and the carbon and PLLA fibres.

ACKNOWLEDGEMENTS

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REFERENCES

1 B Chaignaud et al., 'The history of tissue engineering using synthetic biodegradable polymer scaffolds and cells', In: A. Atala, D. Mooney, *Synthetic Biodegradable Polymer Scaffolds*, Birkauser Boston, 1997.
2 L Freed et al., 'Biodegradable polymer scaffolds for tissue engineering', *Bio/Technology*, 1994 **12** 689-693.

3 H Matsumoto and K Fujikawa, 'Leeds-Keio artificial ligament: A new concept for the anterior cruciate ligament reconstruction of the knee', *Keio J of Medicine*, 2001 **50**(3) 161-166.

4 W Saltzman, 'Cell interactions with polymers', In: R. Lanza, R. Langer, W. Chick, *Principles In Tissue Engineering*, R.G. Landes Company, 1997.

5 P Ma and R Langer, 'Fabrication of biodegradable polymer foams for cell transplantation and tissue engineering', In: J. Morgan, M. Yarmush, Methods In Molecular Medicine **18**, Tissue Engineering Methods And Protocols, Humana Press Inc, 1999.

6 C M Agrawal and R Ray, 'Biodegradable polymeric scaffolds for musculoskeletal tissue engineering', *J of Biomedical Materials Research*, 2001 **55**(2) 141-150.

7 Y Cao et al, 'Tissue engineering of tendon', In: A. Mikos, et al., *Polymers In Medicine and Pharmacy, Materials Research Society Symposium Proceedings*, Pittsburgh, Pennsylvania, **394**, 1995.

8 D Mooney et al, 'Novel approach to fabricate porous sponges of Poly (D, L-Lactic-Co-Glycolic Acid) without the use of organic solvents', *Biomaterials*, 1996 **17**(14) 1417-1422.

9 B Pourdehyhimi et al, 'Measuring fibre orientation in nonwovens-Part V: real webs', *Textile Res J*, 1999 **69**(3) 185-192.

10 J Klawitter and S Hubbert, *J of Biomedical Materials Research Symp*, 1983 **2**(1), 161.

11 J Hubbell, 'Biomaterials in tissue engineering', *Bio/Technology*, 1995 **13** 565-576.

12 N Lamba et al, 'Cell-synthetic surface interactions', In: Patrick et al, *Frontiers In Tissue Engineering*, Pergamon, 1998.

13 M. Lydon et al, 'Cellular interactions with synthetic polymer surfaces in culture', *Biomaterials*, 1985 **6** 396-402.

14 X Walboomers et al, 'Attachment of fibroblasts on smooth and microgrooved polystyrene', *J Biomed. Mater Res*, 1999 **46** 212-220.

15 J Anderson, 'Biocompatibility of tissue engineered implants', In: Patrick et al, *Frontiers In Tissue Engineering*, Pergamon, 1998.

16 S L Edwards et al, 'Design of nonwoven scaffold structures for tissue engineering of the anterior cruciate ligament', 3rd *Autex Conference*, Poland, 2003.

17 H Wan et al, 'A study of cell behaviour on the surfaces of multifilament materials', *J of Materials Science: Materials In Medicine*, 1997 **8** 45-51.

NEW PROPHYLAXIS METHOD OF CHILDREN'S TEETH CARIES

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ABSTRACT

In order to reduce the prevalence and intensity of teeth caries basic stomatological diseases and parodont, we have developed and patented "Anticaries gel with the additive sericin", natural albuminous glue, using silk thread instead of chemical addition "chlorgeksidin".

Silkworm's silk trap gland is allocated fibroin - making an internal part of a silk filament-yarn and sericin (albuminous glue) environmental fibroin. Sericin dissolves in hot water and there are various ways of obtaining it.

For an estimation of anticaries and bactericidal action of anticaries gels, an inspection of forty-four 12 year old children was carried out. As the objective of tests was to determine anticaries action of gel the following parameters were chosen: change of permeability of enamel's demineralization center (EDC) and dynamics of speed changing of saliva secretion (SSS). Bactericidal and anti-inflammatory properties of the additive in anticaries gel were estimated on changing of oral cavity microflora.

Index PMA decrease was achieved in 42 with up to 26% after the end of the treatment, and also absence of irritating effect on bodies of oral cavity confirmed anti-inflammatory action and good bearable anticaries gels with the albuminous additive.

Bactericidal effect of the sericin additive estimated on presence peptostreptococcus and lactobacillus. Significant decrease of their quantitative contents testified to activity of medical gel influence on similar kind of bacterium. Thus, clinical supervision on 44 patients' caries have shown good results.

The sericin additive in anticaries gel is nontoxic, as is a natural product of albuminous origin and at its use does not require any additional precaution. Preparation of anticaries gel with the additive is accessible to any drugstore or laboratory that allows recommending it for wide application in clinical stomatology, especially in case of allergic reactions to chemical preparations and a pathology of gastroenteric path.

"New anticaries gel with sericin additive" caries prevents bactericidal effect and lowers inflammatory gum reaction, suppresses growth of bacteria of type lacto and peptostreptococcus.

Application of a natural product-sericin as the additive does not cause allergic reactions allergy sufferer. Replacement of the chemical additive "chlorgeksidin" with a natural product "sericin" does not disturb physiological balance of oral cavity flora and activity of salivary enzymes.

Since the ancient times, in the field of medicine, different plant origins have been used for treatment of stomatologic disease. Components of cocoons of a silkworm are among them. 'The Canon of a medical science' by Avitenna in the paragraph 'General reasoning on treatment of teeth and dental medicine' it was also stated "that the best dental medicines are those which alongside with drying and absorption add a moisture shine, moderately dissolve surpluses if they have directed to a tooth, and does not give a matter to filter in a tooth. That is a rose and its seeds, pearls, a barley flour, bast mulberries...".

According to the scientific data teeth caries and mucous membrane are amongst the most widely spread diseases among children. There are different ways of prevention and treatment of these diseases.

With the aim of reducing the prevalence and intensity of the basic stomatologic diseases of caries of teeth and mucous membrane, we have researched and patented anti-carious gel with syritzin addition of natural albuminous glue, one of the components of silk string, instead of the chemical «hlorheksidin» addition. From silk separating gland of a silkworm fibroin is allocated which makes up an internal part of a silk string and syritzin, environmental fibroin.

Syritzin has the total exit of aminoacids it makes up about 88%, it is easily dissolved in hot water, and there exists various ways of obtaining it. For estimation of anti-carious and bactericidal action of gel with syritzin addition, an inspection of forty-four 12 year old children was carried out. For the comparison of the results of estimation of our research the same number of children were taken which was considered as control group.

As the objective of determining anti-carious action of gel, the following parameters were chosen:

- change of permeability of nidus demineralization enamels;
- dynamics of speed change saliva secretion;
- intensity of caries gain of permanent teeth.

Bactericidal and anti-inflammatory properties of the addition in anti-carious gel was estimated according to the change of micro-flora of an oral cavity. Anti-carious gel was used as two-single applications per day on a surface of teeth and a mucous membrane of an oral cavity with the duration of 3 minutes manipulation. It was continued for one month.

Dynamics of quality indicators of change of an oral cavity was carried out every other month and every 6 months during 2 years. The results of the research were processed with the help of the method of variational statistics and were compared with the results of the control group. Anti-carious effect of the proposed gel with syritzin addition is confirmed on the basis of three criteria:

1. At the initial teeth caries the stage of nidus demineralization enamel, enamel becomes more penetrating for large molecular connections, including dyes as well. A degree of intensity of spots colouring was determined on L.A.Aksamit's method. As dye for diagnostics of defeat of teeth enamel 2% a solution metilen blue was applied (see Fig.1).

Early spots carious colouring of 2% metilen blue valid enamel demineralization of a teeth is as intensive, as caries enamel defeat is deeper. Application of anti-carious gel with syritzin addition during research reduces interest of penetrating of dye in of nidus demineralization enamel and decrease its colouring.

2. It was noted that in cases of carious disease saliva secretion speed is reduced. The saliva and an oral liquid are condensed and badly wash teeth surfaces, as a result the quantity of dental adjournment is increased on a tooth surface and there appears a risk of high defeat interest (see Fig.2).

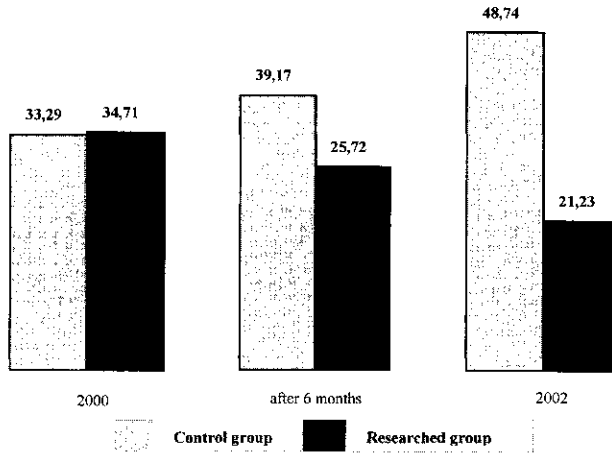

Fig. 1 Changes of Colouring Intensity of the White Spots

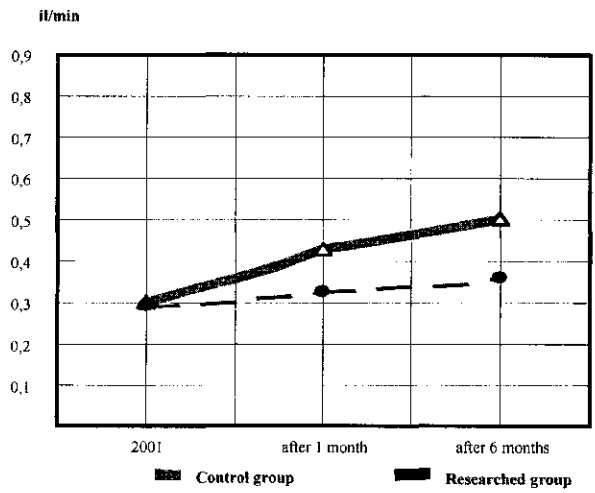

Fig. 2 Change of the Saliva Secretion

Increase of the saliva secretion speed under the influence of the anti-carious gel with syritzin addition testifies to the fact that the saliva liquidises and good washing of teeth surface which in its turn decreases the risk dental adjournment of the formation and increases anti-carious and anti-inflammatory action on teeth and a mucous membrane of an oral cavity.

3. Caries prevention efficiency of the gel was determined every 6 months (see Fig.3).

It is vividly shown in the figure, that index intensity of caries gain of permanent teeth after application gel with syritzin addition in dynamics observation the teeth caries gain of intensity in the researched group is less than in the group of control children.

Anti-carious gel with syritzin addition was estimated as bactericidal action on the growth peptostreptococcus and lactobakterial. Significant decrease of their quantitative maintenance testifies to the activity of gel influence on a bacterium of a similar kind. Thus, clinical supervision on the researched group with the syritzin addition to anti-carious gel use of was well endured by the body.

Our research has shown that syritzin addition in anti-carious gel is nontoxic, as it is a natural product of an albuminous origin and it is not required to carry out measures of additional precaution during its usage. Preparation of anti-carious gel with syritzin addition is accessible in drugstores and laboratories that allows to recommend it for wide application in clinical stomatology, especially in cases of allergic reactions to chemical preparations and pathology of digestive trakt.

We have come to the conclusion that introduction in the structure anti-carious gel with syritzin addition in quantity of 0,005-0,01% has caries preventive effect and lowers inflammatory reaction gum, suppressing bacteria growth of lacto- and peptostreptococcus tupe. As application of a natural product gel – syritzin as the dose not cause any allergic reaction of people who are allergic.

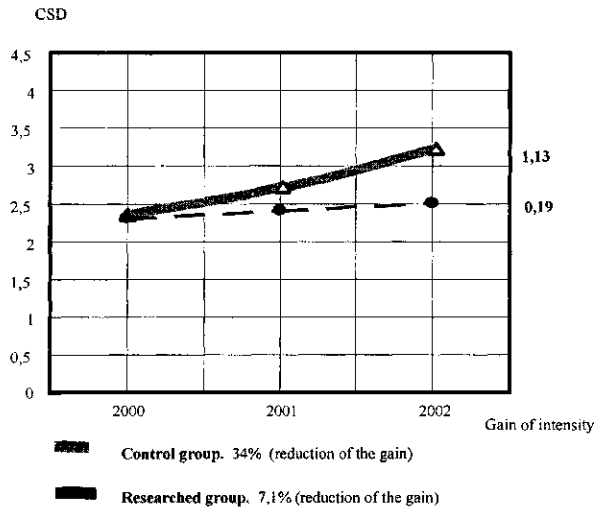

Fig. 3 Gain of Intensity and Reduction of Caries

During the last 2 years we have studied the condition mucous membrane of the oral cavity of the surgery place of the children with a short bridles of the tongue and lips before and after surgery.

As a stitching material we used natural silk and for the control we used artificial silk. Short bridles of the tongue and lips lead to the restriction of the movement or these bodies which in its turn worsens autopurification. The hygienic condition of such

patients is much worse than of the children with normal bridles of the tongue and lips (see Fig.4).

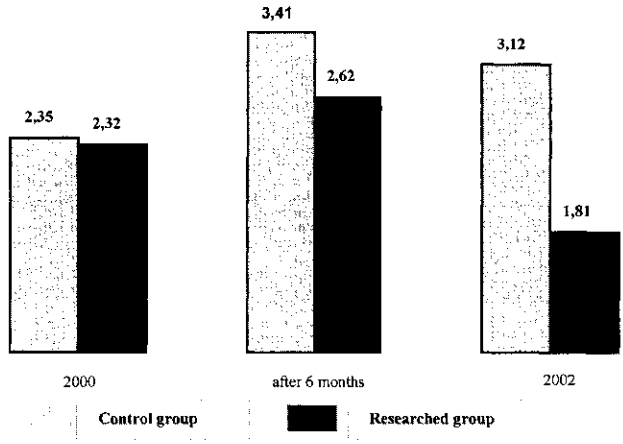

Fig. 4 Avg. Indices of a Hygenic of the Oral Cavity

In the dynamics of observation we came to the conclusion that after imposing a stitching on the 7-8th day swelling with artificial silk is observed aseptical inflammation of a mucous membrane around the stitch. Whereas with natural silk swelling is not observed and aseptically inflammation of a mucous membrane was not noticed, natural silk has property that on the 8-10th day, it tends to be torn away, if the stitch is not very deep.

Properties of natural string: extremely elastic material, high-quality twist, fine results at setting units. A way of radiate sterilization. Period of storage is 3 years.

We suggest the use of natural silk thread in surgical dental practice as it does not cause aseptic inflammation, allergic reaction and is very convenient to use.