Evaluation of Selected Contemporary Biomaterials and Surface Treatments for Soft Tissue Repair Prosthesis

A Thesis submitted by

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In fulfilment of the requirements for the degree of Doctor of Philosophy at Heriot-Watt University, Edinburgh, United Kingdom

School of Textiles and Design March 2010

Abstract

The aim of this project was to determine the best materials and surface treatments for soft tissue repair and to enhance our understanding of material / cell interactions by comparing the response of human cells growing on a selection of currently approved and novel biomaterials. This study focused on comparing the materials and also investigated the effect of modifying the surfaces using gas plasma and other treatments with the aim of enhancing cell growth. In addition, chitosan was studied to examine the reported bacteriostatic effect and promotion of human cell growth.

Chitosan has many properties but this research focused on its reported acceleration of wound healing haemostatic and bacteriostatic properties. To examine the bacteriostatic properties of chitosan, a number of experimental designs were used. The bacteriostatic study led onto a selection of means to incorporate chitosan into/onto some of the biomaterials being tested.

A selection of biomaterials were examined for their ability to support tissue growth in native and surface modified forms (plasma treatment/ chitosan treatment). Cells were seeded on the samples and the growth of the cells was measured at weekly intervals.

The outcome of this research was that the optimal material for soft tissue repair was found to be polyurethane with an ammonia plasma treatment. This can be made into a mesh prosthesis for hernia repair and can be coated with chitosan to inhibit bacterial colonisation if required.

Acknowledgements

I would like to thank Alex Fotheringham for encouraging me and for believing in me. I would like to thank my family for their love and support. I would like to thank my friends in Galashiels for making life fun when I was there. I'd also like to thank my friends at Riccarton for the same reason. I would like to thank Stuart Wallace, Vikash Agrawal, Rodger Spark, Margaret Robson, Andrew McCulloch and all the others that helped with my work in Galashiels. I would like to thank Prof Brian Austin and Dawn Austin for their help in the microbiology lab. I would like to thank Lei Zhang for his tutorial on tissue culture. I would like to thank Anderson Caledonia for their help with sterilising my samples. I would also like to thank Solvay and Rodenburg Biopolymers for their materials. In general I want to thank everybody that has helped me along my way. I would like to make a special thank you for Yifan Wu who has shown extraordinary patience, caring and understanding while I have been working on this project.

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Contents

Definitions

Aliphatic

"Pertaining to any member of one of the two major groups of organic compounds, those with branched or chain structure."

(Dorland, 2009)

Alloplast

"An inert foreign body used for implantation into tissue."

(Dorland, 2009).

Antibiotic

"Antibiotics are a class of natural and synthetic compounds that are able selectively and at low concentrations to destroy or inhibit the growth of other organisms, especially microorganisms."

(Oxford Dictionary of Biochemistry and Molecular Biology, 2000).

Ångström

A unit of length equal to one hundred-millionth of a centimetre (10^{-10} meter).

(The Oxford Dictionary, Thesaurus and Wordpower Guide, 2001)

Apoptosis

"Cell death as a result of an intracellular "suicide" programme. It is a normal and essential event during development generally and within the immune system. Apoptosis does not lead to lysis of cells and thus avoids damage to neighbouring tissue. Alt. Programmed cell death."

(Henderson's Dictionary of Biology, 2008)

Biocompatibility

> *"The ability of a material to perform with an appropriate host response in a specific application"*

(Definitions in Biomaterials, 1986).

This is the preferred definition, commonly referred to as the Williams definition of biocompatibility (The Williams Dictionary of Biomaterials, 1999).

> *"Comparison of the tissue response produced through the close association of the implanted candidate material to its implant site within the host animal to that tissue response recognised and established as suitable with control materials"*

(ASTM International, 2008). This is a specific definition as it refers solely to implanted devices and the local tissue response.

Biomimetic material

> *"Any material that is structurally or chemically analogous to a component of plant or animal tissue and which can be incorporated into any product whose use is based on the characteristics of that tissue component."*

(The Williams Dictionary of Biomaterials, 1999)

Bactericidal

> *"Causing the death of bacteria."*

(Henderson's Dictionary of Biology, 2008)

Bacteriostatic

> *"Inhibiting growth but not killing bacteria."*

(Henderson's Dictionary of Biology, 2008)

Chitin

"Insoluble, linear polysaccharide forming the principal constituent of arthropod exoskeletons and found in some plants, particularly fungi."

(Dorland, 2009)

Cytotoxic

"Attacking or destroying cells."

(Henderson's Dictionary of Biology, 2008)

Extrusion

"To shape a material such as metal or plastic by forcing it through a die. "

(The Oxford Dictionary, Thesaurus and Wordpower Guide, 2001)

Fibroblast

"Flattened, irregular-shaped connective tissue cell, ubiquitous in fibrous connective tissue. It secretes components of the extracellular matrix, including type 1 collagen and hyaluronic acid."

(Henderson's Dictionary of Biology, 2008)

Fistula

"An abnormal passage between two internal organs or from an internal organ to the body surface."

(Dorland, 2009)

Granuloma

"Inflammatory tissue nodule containing proliferating lymphocytes, fibroblasts, giant cells and epithelioid cells, which forms in response to chronic infection or persistence of antigen."

(Roitt and Delves, 1994).

Hernia

"Protrusion of a portion of an organ or tissue through an abdominal opening."

(Dorland, 2009)

Abdominal hernia

"One through the abdominal wall, either a congenital defect or a complication of pregnancy or a surgical incision."

(Dorland, 2009)

Diaphragmatic hernia

"Hernia through the diaphragm."

(Dorland, 2009)

Incisional hernia

"One through an old abdominal incision."

(Dorland, 2009)

Inguinal hernia

"Hernia into the inguinal canal."

(Dorland, 2009)

Cystocele

"Hernial protrusion of the urinary bladder, usually through the vaginal wall."

(Dorland, 2009)

Enterocele

"An enterocele is essentially a vaginal hernia in which the peritoneal sac containing a portion of the small bowel extends into the rectovaginal space between the posterior surface of the vagina and the anterior surface of the rectum."

(Diagnosing and Treating an Enterocele, 1999).

Rectocele

"Hernial protrusion of part of the rectum into the vagina."

(Dorland, 2009)

Mechanotransduction

"Mechanotransduction refers to the many mechanisms by which cells convert mechanical stimulus into chemical activity."

(Katsumi et al., 2004, Liu et al., 1996).

Nosocomial

"Hospital acquired, in relation to infections."

(Henderson's Dictionary of Biology, 2008)

Osteoblast

"Bone forming cell that secretes the bone matrix."

(Henderson's Dictionary of Biology, 2008)

Parastomal

"Para- indicating beside or near. Stoma (stomal) mouth-like opening, particularly an incised opening which is kept open for drainage or other purpose."

(Dorland, 2009)

Plasma (gas)

"Plasma, the 4th state of matter, is a partially ionised gas containing ions, electrons, atoms and neutral species."

(Palmers, 1999)

Prolapse

> *"1. ptosis; the falling down, downward placement, of a part of the viscus. 2. To undergo such displacement."*

(Dorland, 2009)

Uterine;

> *"Downward displacement of the uterus so that the cervix is within the vaginal orifice (first degree prolapse), the cervix is outside the orifice (second degree prolapse), or the entire uterus is outside the orifice (third degree prolapse)."*

(Dorland, 2009)

Pelvic Floor

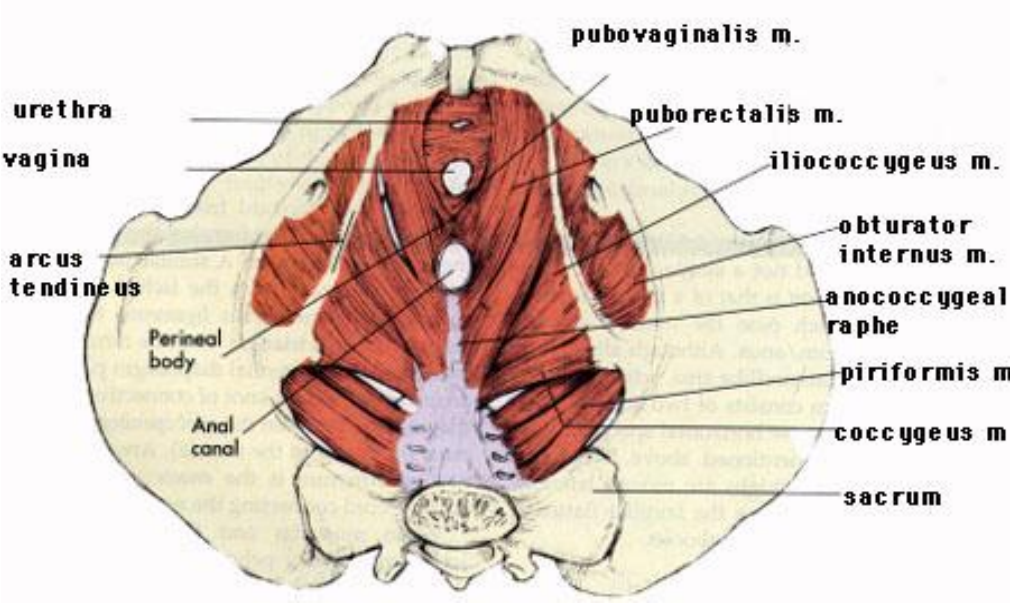


Fig i (Stanford University, 2008)

> *"The pelvic floor or pelvic diaphragm is composed of muscle fibres of the levator ani, the coccygeus and associated connective tissue which span the area underneath the pelvis. The pelvic diaphragm is a muscular partition formed by the levators ani and coccygei, with which may be included the parietal pelvic fascia on their upper and lower aspects. The pelvic floor separates the pelvic cavity above from the perineal region (including perineum) below."*

(Stanford University, 2008)

Kegel exercises

"A Kegel exercise, named after Dr. Arnold Kegel, consists of contracting and relaxing the muscles which form part of the pelvic floor (sometimes called the "Kegel muscles")."

(Wikipedia, 2008).

Seroma

"A seroma is a pocket of clear serous fluid that sometimes develops in the body after surgery. When small blood vessels are ruptured, blood plasma can seep out; inflammation caused by dying injured cells also contributes to the fluid."

(Roitt and Delves, 1994)

Somatic

"Adjective of soma. Soma; The body: The body of an animal or plant excluding the germ cells."

(20th Century Dictionary, 1983)

Stability

"Ability of a substance or material to resist chemical change"

(The Williams Dictionary of Biomaterials, 1999)

Thrombogenicity

"Property of a material which induces and/or promotes the formation of a thrombus"

(Definitions in Biomaterials, 1986)

Thrombus

"A stationary blood clot along the wall of a blood vessel, frequently causing vascular obstruction."

(Dorland, 2009)

Chapter 1 – Introduction

1.1 Research Aims

- To review the advantages and disadvantages of materials used in soft tissue repair and to review potentially alternative materials.
- To investigate in depth a limited selection of these alternative materials.
- To investigate the value of gas plasma treatment on the ability of these materials to support tissue growth in vitro.
- To investigate the reported benefits of using chitosan in relation to medical device applications

One aim of this project was to perform an in depth study into surgical devices used for soft tissue repair (e.g. hernias and prolapses)

The next aim was to evaluate a selection of materials chosen because they are in common use or because they have potential as surgical biomaterials and to explain their advantages and disadvantages, review the potential alternative materials and attempt to demonstrate the efficacy of some alternative materials / surface treatments as tissue scaffolds.

The third aim was to examine how a small selection of surface treatments (gas plasma and chitosan coating) affects their properties as tissue scaffolds (Angelova and Hunkeler, 1999, Chandra and Rustgi, 1998, Guidoin et al., 2000).

By examining how well fibroblasts grew on these materials, biomaterials can be developed that will become incorporated into healthy tissue rather than "scar plates" thus avoiding the negative consequences and therefore this work sought to clarify the potential of a selection of biomaterials based on their ability to support tissue growth in vitro.

In addition to this core body of work, this project aimed to examine the role chitosan can play in biomaterials. By examining the bacteriostatic effect of chitosan and techniques to incorporate chitosan into biomaterials, it was postulated that the biomaterials would incorporate the benefits of containing chitosan, while retaining the properties of the material the chitosan is combined with.

1.2 Current Situation

Polymers are a promising class of biomaterials that can be engineered to meet specific end-use requirements (Angelova and Hunkeler, 1999). They can be selected according to key "device" characteristics such as mechanical resistance, degradability, permeability, solubility and transparency but the currently available polymers need to be improved by altering their surface and bulk properties.

There are many examples of materials that have been used for medical implants that have elicited undesired responses. Current mesh prostheses are made of polypropylene (PP), polyethylene terephthalate (PET) or polytetrafluoroethylene (PTFE), though all of them reveal some disadvantages (Klinge et al., 2002a). The extended implantation of alloplastic material in the flexible frame of muscles and fascial tissue is known to cause specific mesh-related complications like restriction of the abdominal wall mobility (McLanahan et al., 1997, Vestweber et al., 1997), induction of intra-abdominal adhesions with erosion of adjacent organs or consecutive fistula formation (Schneider et al., 1979, Fitzgerald and Walton, 1996), to the bladder (Houdelette et al., 1991, Gray et al., 1994, Hume and Bour, 1996), bowel (DeGuzman et al., 1995, Kaufman et al., 1981, Soler et al., 1993, Miller and Junger, 1997), blood vessels (Schumpelick and Kingsnorth, 1999) and ductus deferens (Silich and McSherry, 1996). Next to an unavoidable inflammatory foreign body reaction (FBR) the prosthesis usually is embedded into a fibrous scar plate, which is responsible for a considerable shrinkage of the mesh area of about 40% (Amid, 1997, Meddings et al., 1993).

Hernias and prolapses are caused by a weakness or defect in the supportive tissues that contain the bodily organs (Morris-Stiff and Hughes, 1998). A hernia (also called a rupture), is a general term referring to a protrusion of a tissue through the wall of the cavity in which it is normally contained. In more specific terms, hernia is usually used to describe a protrusion of the abdominal contents through the abdominal wall. This is usually treated surgically by the implantation of a polypropylene mesh over the defective part of the abdominal wall.

A prolapse is a type of hernia that occurs exclusively in women (Creighton and Lawton, 1998). It is characterised as a failure in the pelvic floor, causing the descent of the uterus. This often presents itself as stress incontinence or in more severe cases, the uterus can descend so far that it protrudes through the vagina. Treatments for prolapses can range from pessaries (which act to provide internal support for the uterus), the implantation of a 'sling' to support the urethra, to hysterectomies (the complete removal of the uterus and ovaries)

1.2.1 Hernia repair

Abdominal Hernia

Abdominal wall hernia repairs are performed over 990,000 times each year in the USA, which makes it second only to cataract procedures, the most common surgical procedure performed in the USA (Rutkow, 1997).

Although surgical techniques in hernia surgery have improved, recurrence used to be a common complication (Engelsman et al., 2007). Therefore, the idea of increasing the strength of the abdominal wall by implanting a mesh was explored with the introduction of a polypropylene (PP) mesh in 1962 by Uscher (Uscher, 1962).

The strength of the abdominal wall depends on the collagen fascia layers, which are the structures to be replaced by a mesh (Engelsman et al., 2007). From a mechanical point of view, abdominal wall implants should become an integral part of the

abdominal wall. This requires complete incorporation of the mesh into the fascial margins of the defect. In the repair of abdominal wall defects, surgical meshes can either be placed fully intra-abdominally (on the surface of the peritoneal lining) or in between different anatomical layers of the abdominal wall. In both situations, the aim of the treatment is to consolidate a musculo-fascial defect without tension on the surrounding tissues.

The most common mesh material used for hernia repair is still polypropylene (PP), although there are alternatives. Trostle et al (Trostle, 1994) mentions polypropylene, expanded polytetrafluoroethylene (ePTFE) polyethylene terephthalate polyglactin 910 (PET) and polyglycolic acid (PGA). These materials vary from rigid strong non-absorbables like PP, to moderately strong very pliable absorbables like PGA.

Vaginal Vault Prolapse

Women face an 11% lifetime risk of surgery for pelvic organ prolapse or urinary incontinence (Olsen et al., 1997). Prolapse and prolapse related conditions account for nearly a quarter of women waiting for routine gynaecological surgery (Creighton and Lawton, 1998). The condition is rarely life threatening but can cause considerable discomfort and stress. Patients with pelvic prolapses commonly have a general state of 'pelvic relaxation', with stress incontinence and some degree of vaginal prolapse coexisting in many patients (Cespedes, 2002). These prolapse conditions include urethral hypermobility, cystocele, rectocele, enterocele and uterine prolapse.

The pelvic floor acts as a support for the pelvic organs and a prolapse occurs when this support fails due to a weakness in the musculo-fiberous tissue (Creighton and Lawton, 1998). The main support for the pelvic viscera is provided by a group of muscles collectively known as the levator ani (Cespedes, 2002). An intact pelvic floor allows the pelvic and abdominal viscera to 'rest' on the levator ani, significantly reducing the tension on the fascia and supporting ligaments. The pelvic ligaments are not true ligaments and are simply condensations of endopelvic fascia covering the

pelvic structures. The vagina can be anatomically divided into the proximal, middle and distal regions. The proximal segment is also called the vault or cuff and is stabilised by the cardinal and uterosacral ligaments. Uterine and vault prolapse are associated with damage to these supportive structures.

Treatments recommended for the different types of prolapse depend on the severity of the condition. Preventative treatments include strengthening of pelvic floor muscles using Kegel exercises (Visco and Figuers, 1998). Cespedes (Cespedes, 2002) mentions that in mild cases of asymptomatic prolapse in which no other procedures are anticipated, the patient will not require surgery. For the elderly patient with severe total vault prolapse who no longer desires sexual intercourse or in whom a short procedure is required because of medical conditions, a vaginal closure or colpocleisis can be performed.

Common techniques available for uterine and vaginal suspension (transvaginal procedure) require drawing each side of the fault together causing restriction of movement. The concept of tension free surgery (the use of a mesh) avoids the need to draw the two sides together and leads to improved wellbeing for the patient with little to no restriction on their movement but the complications must be addressed.

For the repair of vaginal vault prolapses, one of the popular techniques is to suspend the vaginal vault by attaching it to the sacrum using a mesh or cadaverous fascia. This procedure is ideal for young women with severe vault prolapses wishing to retain their fertility or wishing to maintain their sexual activities. Transabdominal suspension using a mesh or cadaverous fascia is a relatively morbid procedure with results comparable to a transvaginal procedure (Nichols, 1991, Kovac and Cruikshank, 1993).

There are other techniques involving permanent suturing of the uterus to alternative support structures, but they are not much better (Cespedes, 2002). The choice of

technique is a difficult one and can dramatically affect the quality of life for the patient.

1.2.2 Complications

To reduce complications, first one must analyse them and the mechanisms behind them. Klosterhalfen et al (Klosterhalfen et al., 1998) report that while there are undisputed advantages to using polypropylene meshes, reports of complications after implantation are increasing. Serious complications such as perforation and fistula formation are rare but minor and local complaints such as seromas, misfeelings and decreased abdominal wall mobility are observed in about half of the patients. A recent paper (Steele et al., 2003) showed complications in 36% of patients from a population of 58 patients requiring Parastomal hernia repair, with complications including recurrence (26%), surgical bowel obstruction (9%), prolapse (3%), wound infection (3%), fistula (3%) and mesh erosion (2%). No patient required extirpation of the mesh. Of the 15 patients with recurrence, 7 underwent successful repair for an overall success rate of 86%.

Morris-Stiff et. al. (Morris-Stiff and Hughes, 1998) mention that despite the reported low tissue reactivity and long term maintenance of tensile strength associated with PP mesh, they had seen four patients in whom these properties failed during long term follow up of forty patients in a single unit. The four patients included three with dense adhesions (one with severe infection) and one with primary mesh failure, all requiring re-operation. It is mentioned that complications of non-infected wounds are notably absent from current literature (1998) and suggests that these complications may occur more often than is reported. The reasons proposed are short periods of follow up, a lack of association between the complications and the mesh or reluctance to report them.

PP and PET fibre meshes can cause tissue damage including; reduced mobility, severe adhesion formation causing bowel obstruction, subsequent erosion and formation of fistulas when placed in direct contact with the intestine and the

incorporation of the prosthesis into a fibrous scar plate which in turn can cause the mesh to shrink up to 40% (U.Klinge et al., 2002, Law and Ellis, 1988, Klinge et al., 2002b). Therefore, its application is avoided when the mesh may be in direct contact with the intestines.

In addition to problems with adhesion, when polymeric biomaterials are implanted within the body, the immune system responds. This reaction is caused by a foreign body reaction (Coleman et al., 1974, Marchant and Anderson, 1986, Marchant et al., 1986). Foreign body reactions are characterised by an initial acute inflammatory reaction. A chronic granulomatous (see Granuloma in Chapter 1) tissue reaction may persist, even after encapsulation has occurred. The foreign body reaction seems to be induced by continuous chemical or mechanical stimuli arising from the biomaterial implants (Coleman et al., 1974). Morphological analysis of this reaction reveals the presence of a large number of macrophages, which generally attempt to phagocytose the material. Usually the foreign body is much larger than individual macrophages and is not easily degraded. Some of the macrophages then merge their cytoplasm to become multinucleated giant cells also called foreign body giant cells. If the foreign body cannot be degraded by phagocytes, granulation tissue is formed to isolate the implant from the rest of the body tissues. The foreign body reaction may be assessed in a semi-quantitative way by the enumeration of inflammatory cells, namely, polymorphonuclear leukocytes (PMN) and activated macrophages or giant cells found either at the surface of the implanted biomaterials in the inflammatory exudative fluid elicited by implants (Coleman et al., 1974, Marchant and Anderson, 1986, Marchant et al., 1986).

The contribution of phagocytic cells to the foreign body reaction may involve two closely related mechanisms (Vaudaux et al., 1994). In the first, the neutrophils or macrophages phagocytose the smaller fragments of the biodegraded or corroded metallic or plastic implants. These fragments cannot be degraded further and they may persist intracellularly in the neutrophils or macrophage for a prolonged period of time or may be ingested by other phagocytes if cell death does occur. In the second reaction also called “frustrated phagocytosis,” phagocytic cells are confronted with

foreign particles such as nylon wool, glass, cotton, polysulfone fibres, polystyrene or polypropylene materials too large to be ingested (Henson, 1971, Johnston and Lehmeyer, 1976, Klock and Bainton, 1976, Wright and Gallin, 1979, Yanai and Quie, 1981). Phagocytes coming into contact with this non-phagocytosable foreign material become permanently activated in a way similar to phagocytes containing the smaller fragments of non-degradable foreign particles; each kind of phagocyte may separately or in concert secrete or passively release several important inflammatory mediators (Coleman et al., 1974, Marchant et al., 1986), including acidic or neutral hydrolases, activated complement components, tumour necrosis factor (TNF), interleukins, prostaglandins, plasminogen activator and coagulation factors (Vaudaux et al., 1994). The respective roles and the relative importance of these secreted factors in the control and maintenance of acute and chronic phase of the inflammatory response to implants are not yet well defined (Baggiolini, 1982, Coleman et al., 1974, Gallin, 1984).

1.3 Prosthesis Related Infections

Infections are one of the most frequent and serious complications associated with indwelling medical devices (Vaudaux et al., 1994).

Infections of biomaterial applications, including surgical meshes, are especially troublesome as a biofilms can be formed on the mesh. Biofilms are formed when micro-organisms colonise a surface and excrete a polysaccharide matrix. Micro-organisms in this biofilm are protected against the host immune response and antimicrobial attack (An and Friedman, 1998, Zimmerli et al., 1984). The body continues to try to clear the microorganisms and this ends up causing damage to the surrounding tissue. This will often lead to major complications which can be potentially life-threatening and will in the majority of cases result in removal of the mesh (Costerton et al., 1999). Bacteria look for a permanent surface to bind to as it affords them greater protection against the body's immune system, so a non-permanent implant should circumvent that problem.

1.3.1 Incorporation of antimicrobials into medical prostheses

To avoid the problem of biomaterial related infections, one can try to incorporate antibiotic or bacteriostatic compounds into the material. Most published data for antimicrobial textiles and fibres are generated by placing a fabric on an inoculated nutrient agar plate and measuring the inhibition zone (stanford.edu, 2008). This procedure depends on diffusion of the antimicrobial agent in the agar. Further work is usually required to discover the mechanism of the antimicrobial properties. This is required if one wishes to discover if the compound being tested is bacteriocidal or bacteriostatic. The difference between bacteriocides and bacteriostats is subtle. Antibiotics have been widely used and antibiotic pathogens have developed as a result, but the inhibition of growth using bacteriostats is less common and could be used as a prophylactic alternative to antibiotics. With an appropriate antimicrobial incorporated into a biomaterial, it is anticipated that this would significantly reduce the chances of post operative infection and potential biofilm production

1.4 Tissue Engineering

The desired effect of any tissue engineering is to restore, maintain or improve the function of human tissues.

The tissue engineering paradigm is to isolate specific cells through a small biopsy from a patient, to grow them on a three-dimensional biomimetic scaffold under precisely controlled culture conditions, to deliver the construct to the desired site in the patient's body and to direct new tissue formation into the scaffold that can be degraded over time (Lee and Mooney, 2001). Tissue engineering (TE) merges many aspects of engineering and life sciences, aiming towards the primary understanding of cell functions and the advancement of biological substitutes (Wiria et al., 2007).

Degradable materials are less susceptible to infection and intend to cause less of a foreign body response (Badylak et al., 2001). However, the lack of strength over time is a concern for certain clinical applications where adequate tensile properties are necessary and required.

"Tissue engineering concepts of producing a lattice for the ingrowth of cells *in vivo* to lay down the appropriate matrix have been used very successfully for the skin and for the repair of the facia in hernias. The approach used by researchers has been to assume that cells and their accompanying matrix need a scaffold to enter, adhere to and proliferate in an ordered manner. The three features of the tissue-engineered scaffold are the overall architecture and porosity, the fibre morphology and the surface chemistry. The use of knitted polyester meshes with pore sizes many orders of magnitude larger than the repair matrix requires can result in a tissue response that is inadequate. Pore sizes of between 10-50 μm and overall porosity of 85-90% with a multifilament fibre yarn with fibre diameters of 1-10 μm appear to be the most ideal for tissue ingrowth." (Minns, 1999)

Other papers claim slightly different values for the "optimal" pore size. In a recent paper, the author conducted a study where he developed polycaprolactone scaffolds with varied pore sizes using a centrifugation method and therefore studied how different pore sizes suit different applications. The scaffold section with 380–405 μm pore size showed better cell growth for chondrocytes and osteoblasts, while the scaffold section with 186–200 μm pore size was better for fibroblasts' growth. The scaffold section with 290–310 μm pore size showed faster, new bone formation than those of other pore sizes (Oh et al., 2007).

In cartilage tissue remodelling in response to mechanical forces, (Grodzinski et al., 2000) Grodzinski, mentions recent studies which suggest that mechanotransduction is critically important *in vivo* in the cell mediated feedback between physical stimuli and the resulting macroscopic biomechanical properties of the tissue. This should be an important consideration when selecting materials, especially degradable materials intended to regenerate damaged tissue.

Another important consideration, often overlooked, is how the regenerating tissue reacts with the prosthesis. The material used should elicit no negative effects on the growing cells. This can be difficult to measure, but testing the cells for Heat Shock Protein (an indicator of macrophage stress) (Henze et al., 1996) and produced by

other cells growing in a stressful environment) is potentially an effective way of quantifying cellular stress. A simpler approach may be to measure how well cells grow on a sample. This approach may not be so accurate, but should indicate a cellular preference for a particular material/ surface that can then be followed up by more elaborate testing.

1.5 Reasons for Improvement

These papers indicate the need for a new type of mesh implant for the repair of incisional hernias and vaginal prolapses. In discussion with Dr Fotheringham (PhD Supervisor) and Dr Browning (Gynaecologist), it became apparent that instead of trying to produce a new permanent implant, the market would soon be ready for an absorbable implant that could encourage the patient's tissue to repair the fault and then dissolve so there is no surface for bacteria to adhere to and the problem adhesions and encapsulation would be avoided, as these are a host response to a foreign material placed within the body. With permanent implants, the immune system takes the material as a threat and when bacteria bind the implant and bind to it, this amplifies the problem. By having an implant that is constantly dissolving, the problem of the macrophages trying to engulf the entire implant is avoided. Instead, the immune system is able to encapsulate the small fragments of dissolving material. The bacteria do not have a permanent surface to adhere to which will reduce the chance of infection at the implant site in the long term. With a permanent implant, even if the surgery is performed perfectly, the chance of infection at the implant site is still dramatically increased.

1.5.1 Niche

There is demand in the medical profession for a new generation of medical implants. They are looking for implants for repairing hernias and prolapses that will avoid the problems that current mesh technologies cannot. This review is one of several indicating the problems associated with the current permanent meshes on the market.

The ultimate solution would be a re-absorbable implant that would encourage new tissue to grow over the implant to eventually replace it, one that would not antagonise the immune system and inhibit bacterial growth/ adhesion. An implant that satisfies these parameters would find many applications.

Therefore a strategy would be to take the body as a template and try to mimic the body's original structure. The main obstacle to determining the characteristics required for such a design is the fact that there is little research into the mechanical properties of the pelvic floor and even less on how the body subconsciously controls it. An implant could be designed to mimic the mechanical properties, but little will be known about how successful it will be without the necessary somatic control. With this in mind, it would be sensible to make sure that this implant will exceed requirements.

Chapter 2 - Material Review

In the process of selecting materials, there are several requirements a biomaterial must meet. The most important of these is biocompatibility. It must not illicit an undesired response when placed within the body. The ideal material should be;

biocompatible

stable

biomimetic

The material should maintain strength as long as required. It should have strength and bear load in a manner homologous with the tissue it is to emulate but it should not be so strong that it restricts the mobility of the patient, or damage surrounding tissue under stress.

In the case of biodegradables for soft tissue repair, one is looking for a material that will transfer load from the device to the tissue as it is regenerating (Grodzinski et al., 2000) so that the repaired tissue will be strong enough when the material has degraded.

In addition, the device should not be prohibitively expensive. Therefore if the medical device cost is kept to a reasonable level, it will be a viable option for more patients and be better placed to compete with its competing products.

The choice of material(s) is of vital importance to the success of an implant but there are so many aspects that need to be examined. The ideal implant should inhibit adverse reactions and bacterial growth/attachment yet promote healthy, controlled tissue regeneration. Unless cloned tissue is used, there is little chance there will be a single material that can emulate the native tissue, therefore a combination of materials and treatments may be necessary.

The materials used in this project shall be a selection of materials that fit three criteria. They will be either 1. Currently approved materials, 2. Available novel materials and 3. Modifications of these materials.

2.1 Potential Materials

The materials selected for this study shall be selected for the following reasons; they should be either currently used as medical prostheses (in the case of the permanent materials), or potentially suitable for medical use (in the case of the resorbable materials) and they should be available to the researcher.

There are two objectives for this study. One is to evaluate a range of permanent and degradable materials as scaffolds for tissue regeneration. The other aim is to evaluate a selection of surface treatments for their ability to enhance biocompatibility and tissue regeneration whilst maintaining their bulk properties.

2.1.1 Material selection

Polymers used as biomaterials can be naturally occurring, synthetic or a combination of both. (Angelova and Hunkeler, 1999)

Naturally derived polymers are abundant and usually biodegradable (Chandra and Rustgi, 1998). Their principal disadvantage lies in the development of reproducible production methods, because their structural complexity often renders modification and purification difficult. Additionally, significant batch-to-batch variations occur because of their 'biopreparation' in living organisms (plants, crustaceans) (Angelova and Hunkeler, 1999) .

Synthetic polymers are available in a wide variety of compositions with readily adjusted properties. Processing, copolymerization and blending provide simultaneous means of optimizing a polymer's mechanical characteristics and its

diffusive and biological properties. The primary difficulty is the general lack of biocompatibility of the majority of synthetic materials, although poly (ethylene oxide) (PEO) and poly (lactic-*co*-glycolic acid) are notable exceptions. Synthetic polymers are therefore often associated with inflammatory reactions, which limit their use to solid, unmoving, impermeable devices (Angelova and Hunkeler, 1999).

With these considerations in mind the next stage is to narrow the field of prospective materials through a process of elimination.

In 'functional assessment and tissue response of short- and long-term absorbable surgical meshes' (Klinge et al., 2001) it is mentioned that while non-absorbable devices usually tend to produce fistulas in direct contact with the bowels, the interposition of short-term absorbable meshes result in large incisional hernias in almost all cases. The study investigated the functional and histological consequences of a short-term polyglactin 910 (Vycryl®, loss of 50% of its mechanical stability within three weeks) and a long-term absorbable mesh polylactide (LTS, preserved >50% of its stability for over one year). The PG-mesh initially revealed a pronounced inflammatory reaction and a significantly increased formation of connective tissue in the interface mesh/recipient tissues correlated to an increased stiffness of the abdominal wall compared to the sham-group (The sham-group consists of incisions sutured together with no implanted mesh). However, a loss of mechanical stability and an increase in elasticity could be detected three weeks after implantation, which may be explained by the rapid absorption of the mesh material. In contrast to PG, the LTS mesh indicated a decreased but persisting inflammatory reactions in the interface mesh/recipient tissues and significantly reduced induction of connective tissue. Although the formation of scar tissue was diminished compared to PG, the LTS mesh preserved its mechanical stability after 180 days. The results indicate that the frequent development of incisional hernias with short-term absorbable meshes (PG) might be due to the decreased mechanical stability and dilation of the newly formed connective tissue after 2-3 weeks. Moreover extensive scar tissue formation may promote adhesion formation.

To decide which of the many biomaterials to study, one must make out a list of potential materials and weigh up the criteria for and against. This will not be a complete list, as there are many exotic biomaterials being developed and therefore it will contain materials that are readily available.

Natural Polymers

Proteins and protein based polymers

Collagen

Collagen is expensive and suffers from large batch-to-batch and source-to-source variations typical of natural extracts (Angelova and Hunkeler, 1999). Collagen would be an ideal material if complications such as variation and potential for disease transfer could be circumvented. In addition, tissue sources that have origins from other humans or animals remain problematic mainly due to immunogenic responses by the patients (Shin et al., 2003).

Koob (Koob and Hernandez, 2002) published research data on the modification of native collagen to produce re-synthesised collagen fibre. The outcome of this work was a biologically based tendon bio-prosthesis with mechanical properties equivalent to native tendon. Ultimate tensile strength of the NDGA cross-linked fibre was greater than that of native tendon, while the elastic modulus and strain at failure were comparable to those of tendon fibres.

Polysaccharides and derivatives

Chitin / Chitosan

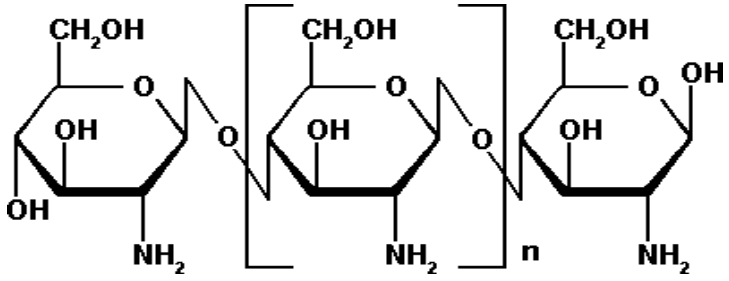


Fig 2.1 - Chemical formula of chitosan in Haworth's projection (Murúg, 2007).

Chitin is one of the most abundant natural amino-polysaccharides and is estimated to be produced annually almost as much as cellulose. Its immunogenicity is low, in spite of the presence of nitrogen (Majeti and Kumar, 2000). Its purity can vary as a result of its origin (e.g. (crab shell chitin = low purity, Squid chitin = higher purity.) It can also vary in molecular weight (e.g. squid chitin = high molecular weight) and these factors can affect the properties of the chitin. Another variable for chitin is the degree of deacetylation. Chitosan is a deacetylated form of chitin and by varying the degree of deacetylation, its biodegradability and solubility can be modified. Chitosan biodegrades hydrolytically and this is enhanced by the presence of lysozyme (Lee et al., 1995). The susceptibility to lysozyme of chitin derivatives is controlled by the degree of acetylation at the C2-position and/or by the introduction of various substituents at the 6-0-position of the N-acetylglucosamine residue (Nishimura et al., 1985).

Chitosan has many possible applications, but the applications of most interest for this study are its tissue culture properties and its bacteriostatic effect. Chitosan has some level of antimicrobial activity and fibres made from chitosan are available in the marketplace (stanford.edu, 2008). Coatings of chitosan on conventional fibres or films appear to be a more realistic prospect for development of this material (Broughton et al., 2001).

Chitosan In relation to medicine

In a paper by Hwang (Hwang et al., 2000) it is mentioned that nitric oxide (NO) contributes towards cytotoxicity in cell proliferation during inflammation of wound healing. NO is a highly reactive free radical and is employed by the immune system to respond to inflammatory agents such as LPS (lipopolysaccharide derived from bacterial cell walls) and interferon-gamma that activate macrophages and stimulate them to produce NO. Chitin and chitosan show a significant inhibitory effect on NO production by the activated macrophages. This would help explain the beneficial role that chitin and the deacetylated derivatives have on wound healing.

Deacetylated chitin derivatives such as 70% deacetylated chitin (DAC-70) and 30% deacetylated chitin (DAC-30) have potent immunological activities for activation of peritoneal macrophages *in vivo*, suppression of Meth-A tumour cells in syngenic BALB/c mice and stimulation of non-specific host resistance against *Escherichia coli* infection in mice (Nishimura et al., 1984). Chitin and chitosan are also effective for the protection of host against infection with Candida *albicans* and Staphylococcus *aureus* and against growth of Ehrlich and Sarcoma 180 ascites tumour (Suzuki et al., 1982, Suzuki et al., 1984). All deacetylated derivatives of chitin are reported to enhance the activity of natural killer (NK) cells as well (Nishimura et al., 1985).

Because chitin and its deacetylated derivatives do not provoke an unfavourable immunological response, chitin derivatives have been suggested for bandages, sutures and other items placed in the human body (Brown, 1999) although purity will be an issue in these applications (Broughton et al., 2001).

One issue with using chitin and chitosan for medical devices is the difficulty in producing useable fibres. The poor tensile strength of chitosan fibres, especially in the wet state, is a key deficiency (Notin et al., 2006). This is part of the reason why there are so few products using chitin or chitosan on the market with the exception of wound dressings (Niekraszewicz, 2005, Ong et al., 2008). This is being addressed by scientists working on novel extrusion techniques or via the use of additives during extrusion (Notin et al., 2006, Qin et al., 2002).

Synthetic Polymers

Polyanhydrides

Polyanhydrides are a group of polymers with two sites in the repeating unit susceptible to hydrolysis (Angelova and Hunkeler, 1999). Polyanhydrides are useful materials for drug delivery. The degradation rates can be altered with changes in the polymer backbone. Aliphatic polyanhydrides degrade within a few days while aromatic polyanhydrides can degrade slowly over a period of several years.

Aliphatic polyesters

Almost the only high molecular weight compounds shown to be biodegradable are the aliphatic polyesters. The reason for this is the extremely hydrolysable backbone found in these polyesters (Angelova and Hunkeler, 1999).

Poly-ε-Caprolactone (PCL)

Poly(ε-caprolactone) (PCL) has been studied as a substrate for biodegradation and as a matrix in controlled-release systems for drugs and its slow rate of degradation *in vivo* makes it suitable for devices with longer working lifetimes (1–2 years) (Chandra and Rustgi, 1998).

This material is primarily being developed as a bone substitute for use in maxillofacial reconstructive surgery. However, it could be adapted to other areas where bioabsorbable composite materials may be used (Corden et al., 2000).

In vitro biocompatibility of both the in situ polymerised PCL and commercially available PCL (Solvay's CAPA 6400) material has been assessed using osteoblasts derived from human craniofacial bone cells. The material is highly biocompatible with these cells which will attach and spread on both the PCL types.

The main factor influencing cell behaviour seems to be the surface topography of the polymer samples (Corden et al., 2000). A tendency of cells to group, showing zones with more cellular density, was observed on PCL films, although these nuclei of growth disappeared when cultures reached confluence (Serrano et al., 2005).

Polyglycolic acid

Polyglycolic acid or PGA is the simplest linear aliphatic polyester, with repeat units –OCH_2CO- and is a readily degradable highly crystalline polymer used for sutures and other implantable devices (The Williams Dictionary of Biomaterials, 1999).

The advantage of poly-glycolic acid is the degradability by simple hydrolysis of the ester backbone in aqueous environments such as body fluids. Furthermore, the degradation products are ultimately metabolized to carbon dioxide and water or are excreted via the kidney (Chandra and Rustgi, 1998).

Although poly-glycolic acid is a commonly used biomaterial in medical devices, it is a short term resorbable polymer which eliminates it as a structural component of a tissue repair mesh for connective tissue, although it is often used as a copolymer to increase the degradation rate.

Poly-l-lactic acid

PLA is a relatively hydrophobic linear aliphatic polyester, with repeat units $OCHCH_3CO$. PLA has similar properties to polyglycolic acid except that degradation occurs more slowly. PLA exists in two stereoregular forms, D-PLA and L-PLA and in the racemic D,L-PLA (The Williams Dictionary of Biomaterials, 1999).

Polymeric scaffolds including synthetic materials such as poly(L-lactic acid) have attracted significant interest in the tissue engineering community as a consequence of

their biocompatibility, ease of processing into three-dimensional structures, their established safety as suture materials and the versatility that they offer for producing chemically defined substrates for graft matrices (Kanczler et al., 2007).

Aromatic polyesters

Polyethylene terephthalate is an aromatic polyester (aromatic polyesters are often just termed polyester). The sample used in this project was donated by Vascutek Ltd in the form of an arterial prosthesis and has therefore been tested to ensure its biocompatibility and anti-thrombogenicity.

Aliphatic-aromatic polyesters

Solanyl Flexibilitis component (or Eastar Bio GP copolyester)

Aliphatic-aromatic co-polyester the name of Solanyl® is derived from Solanum Tuberos. The polymer is made from by-products of potato processing, the potato peels (Rodenburg Biopolymers, 2004).

Having seen this material, the Flexibilitis grade appears to have very good mechanical properties and it would be interesting to find out how human cells react to it. For the purpose of this work, it shall be referred to as Solanyl.

Polypropylene

Polypropylene is a thermoplastic homopolymer, made by the chemical industry and used in a wide variety of applications including medical devices such as Marlex® which is a commercially available hernia repair patch. Polypropylene has many advantages and disadvantages but the material has a long history in medical devices and therefore it is important as a control. Experiments were conducted in this thesis to determine if tissue response could be improved.

PTFE (Polytetrafluoroethylene) (Teflon®)

PTFE is a chemically inert homopolymer, with a very low coefficient of friction (Young and Lovell, 1991) and as such, has found numerous applications in biomedical devices. PTFE is commonly used in vascular grafts and tendon repair, both applications where low friction and hydrophobicity are an advantage, but this is a disadvantage when looking for cell adhesion and tissue regeneration. Therefore this material is not ideal for this study as it is so hydrophobic.

Thermoplastic polyurethanes

Polyurethanes are a large family of polymers in which urethane bonds are formed in the backbone of molecule chains by the reaction between a polyol and an isocyanate and can be either thermoplastic or thermosets.

Among synthetic materials, polyurethanes have been considered to be the most suitable material in various biomedical applications, which is connected to their biocompatibility, biodegradability and controlled microstructure and properties (Corneillie et al., 1998). They also have excellent mechanical properties which makes them well suited to biomedical applications.

Carbon fibre

Carbon fibre initially appears to be a very suitable material as described by R.J. Minns (Minns, 1999). In his paper, Tissue engineered Synthetic Scaffolds for Tissue repair– a textile approach to implant design he states that individual carbon fibres appear to present an attractive surface, morphologically and chemically, to the attachment of fibroblasts which eventually produce a collagenous framework within the implant scaffold at the sites desired.

During questioning at the MedTex conference in 2003 (Bolton, UK), when R.J. Minns was presenting, Royston Dawber raised an issue, mentioning that he was aware of an autopsy on a 60 year old woman who had died and it was discovered that

a carbon fibre from a tendon repair had migrated through her body and been discovered in her brain. This news was enough to discount this material as a potential prosthesis for this project. Incidentally, there have not been any recent papers proposing the use of carbon fibre for soft tissue repair.

2.1.2 Chosen Materials

Polypropylene

Polypropylene was chosen partially because it is widely used for medical prostheses, therefore would act as a reference material. In addition it would be of value to see if altering its surface properties would improve the tissue reaction.

Polyurethane

Chosen for its biocompatibility, biodegradability and mechanical properties.

Polyester (Vascutek)

This material is an example of a vascular prosthesis. As it is currently used in surgery, examining the way cells proliferate on this material and how the cells react to the material is of great interest. This was used as the gold standard control and to demonstrate how well cells should grow on a biomaterial. It was also examined to see if the cell material interaction can be improved by plasma treatment.

Poly-ε-Caprolactone 6400

Chosen because it is biocompatible, flexible, biodegradable and has a large body of published work relating to medical use.

Solanyl

Chosen for its mechanical properties, biodegradability and lack of published work.

Poly lactic acid

Chosen because it is biocompatible, biodegradable and has a large body of published work relating to medical use.

In addition to the reasons stated above, another selection criterion for these materials was that they were available in sufficient quantities to perform this research project.

2.2 Potential Treatments for Materials

2.2.1 Chitosan/ chitin coating

As mentioned earlier by R. Broughton (Broughton et al., 2001) coatings of chitosan on conventional fibres or films appears to be a more realistic prospect for development of this material although development of a fibre would be very useful. To make this idea a commercial reality, a method needs to be developed of applying a uniform coating of chitosan to a material. There is a concept of spray application that could have many applications in the medical sphere. Some materials may need surface alteration to make the material wetable before any lasting chitosan coating can be applied. In a preliminary study into the effect of chitosan-coated material on MRSA and Staphylococcus *epidermis*, the coated polypropylene performed poorly, whilst the chitosan-coated cotton cloth had an observable effect. This indicated that the material needed to be wetable for the chitosan coating to adhere sufficiently to the material to be useful (Method 3c in the results section).

Another potential approach to incorporating chitosan to a polymer would be to use gas plasma to cross link the chitosan to the polymer surface. It would be interesting to compare the various methods of chitosan coating.

2.2.2 Low Pressure Plasma Treatment

Low pressure plasma treatment can be used to alter a materials hydrophobicity / hydrophilicity, sterilise materials without the problems associated with other methods and to erode the surface to enhance roughness of a material (Palmers, 1999). This can be achieved in a reproducible manner by ionising the gas in a controlled and qualitative way within a vacuum vessel (pumped down to a pressure in the range of 10^{-2} to 10^{-3} mbar). The gas is ionised with the help of a high frequency generator. The highly reactive particles react with the surface of the substrate. The gas used can be altered, the power used and length of exposure can be altered to promote the desired effect (ablation, crosslinking, activation or deposition). The formed reactive particles react in a direct way with the surface without damaging the bulk properties of the treated material as the surface modification is limited to the outermost 10 to 1000A (Ångström) of the substrate.

The lifetime of the treated polymer surface can be a concern. A disadvantage of polymer surface treatments is that the modified surfaces undergo surface restructuring with time (Yang et al., 2002) owing to the mobility of the polymer chain in the amorphous regions (Murakami et al., 1998, Kim et al., 2003), which is driven by thermodynamic need to lower the overall interfacial energy of the system (Koberstein et al., 1998).

<u>Oxygen Plasma</u>

Oxygen plasma treatment is an effective means of enhancing the hydrophilicity of a polymer's surface. This enables polymers that would normally be unsuitable for tissue growth to be able to support the attachment of cells. According to Van-Kooten (van-Kooten et al., 2004), the improved wettability of oxygen plasma treated materials was related to improved cell proliferation, increased fibronectin surface coverage and increased expression of adhesion related proteins.

There also appear to be other advantages to oxygen plasma treatment. In adhesion of Pseudomonas *aeruginosa* strains to untreated and oxygen-plasma treated poly (vinyl

chloride) (PVC) from endotracheal intubation devices by K. Triandafillu (Triandafillu et al., 2003) they mention that oxygen plasma treatment has a beneficial effect against the bacterial colonization of a Oxygen plasma treated PVC, reporting a 70% reduction in adhering bacteria although they concede that this reduction is however unlikely to be sufficient to prevent P. aeruginosa colonization of endotracheal intubation devices.

This would be an attractive surface treatment to examine, as it appears to yield promising results. Unfortunately technical problems conspired to make this treatment unavailable for the majority of the materials.

Argon Plasma

Argon is an inert gas, so while it will ablate the surface of the polymer and improve the hydrophilicity, it will not create a functional group on the surface of the polymer.

Ammonia Plasma

It is hypothesised that plasma treatment with ammonia would improve tissue growth along a biomaterial more than argon plasma treatment. This was suggested as ammonia is made of nitrogen and hydrogen, which are the building blocks of proteins (Proteins are built from amino acids and amino acids are so called because they contain an amine group (NH_2)). Therefore it was suggested that a material presenting nitrogen and hydrogen on its surface would mimic a protein and therefore encourage cell binding and greatly enhance its biocompatibility.

Fluorine Treatment

Fluorine is the most electronegative and reactive of all elements (Fessenden and Fessenden, 1990). Treatment of polyester with a solution of fluoropolymer (polyvinylidene fluoride) has been shown to reduce thrombogenicity (Maini, 1999).

Due to this lower thrombogenicity, this biomaterial is now used for vascular prostheses with a diameter of 6mm.

2.2.3 Hyaluronic acid

In a paper by D. Girotto (Girotto, 2003) it is reported that the re-differentiation capabilities of human articular and chick embryo sternal chondrocytes were evaluated by culture on HYAFF-11 and its sulphate derivative, HYAFF-11-S, polymers derived from the benzyl esterification of hyaluronate. Initial results showed that the HYAFF-11-S material promoted the highest rate of chondrocytes proliferation.

2.2.4 Laser pitting

Prof Duncan Hand at Heriot-Watt University in Edinburgh developed a technique using lasers to introduce pits of controllable size into a material (Fotheringham et al., 2004). It was thought that this would be useful for encouraging cells to grow on the proposed implant. This was discussed and while material could be pitted for tissue culture study, the technology was prohibitively expensive and slow in its current incarnation.

2.2.5 Micro-grooves

In a paper by E.T. den Braber (Braber, 1996), planar and micro-textured silicon substrata were produced and made suitable for cell culture by radio frequency glow discharge treatment and media were produced with grooves with widths of 2μm, 5μm and 10μm and depth of 0.5μm. Cell counts proved that neither the presence of the surface grooves nor the dimensions of the grooves had an effect on cell proliferation, although cells grown on the 2μm and 5μm wide grooves were elongated and aligned parallel to the surface grooves. It was also shown that cells on the 10 μm grooves were almost comparable with the control with no grooves. Finally, it was also observed that cells on the micro-textured substrates were capable of spanning the surface grooves.

It was also mentioned that these results contradict the work reported by Green (Green et al., 1994) and Ricci (Ricci, 1994). It goes on to mention that a response to surface topography is dependent on cell type, which would account for the discrepancies between this and other studies.

2.2.6 Chosen treatments for materials

Given more time and resources, one could compare all of these surface treatments and develop treatment combinations but unfortunately, only a few treatments could be analysed, due to the aforementioned limitations.

The treatments chosen were plasma treatment and chitosan coating and a combination of plasma treatment and chitosan coating.

Chapter 3 - Methodology

The experimental studies can be split into two distinct groups. The first is the examination of chitosan as a bacteriostat and the second is the production and testing of biomaterial samples.

3.1 Examination of Chitosan as a Bacteriostat

This series of experiments was designed to examine the bacteriostatic effects of chitosan on common hospital bacteria. The bacteria chosen were methicillin resistant *Staphylococcus aureus* (MRSA 9551) and *Staphylococcus epidermis* (*Staphylococcus epidermis*).

3.1.1 Materials

Nutrient agar (NA)

Nutrient broth (NB)

Plate Count Agar (PCA)

Petri dishes (~10 cm)

Culture bottles (~25ml)

Inoculation loop

Bunsen burner

Scissors

Tweezers

Ethanol (100%)

Distilled Water

Methylene blue

Acetic Acid (2M)

Sodium Hydroxide (2M)

Chitosan (Purisan™ squid chitosan – high molecular weight)

Cotton cloth (unbleached)

Neubauer Improved Haemocytometer (Vol = 1/400 ml per small square)

Incubator (37°C & 20°C)

Autoclave

Gilson pipettes (20µl – 1ml)

Chitosan Materials (Various Production Methods)

All chitosan work was performed using Purisan™ PB-103 squid chitosan, high molecular weight from Sigma Aldrich (made by Technology Resource International Corporation). The 2M acetic acid was made from glacial acetic acid (reagent grade, Acacia).

All of these samples were autoclaved (sterilised) prior to use in the experiment, at 121°C for 15 minutes unless stated otherwise. This produced some discolouration in the chitosan coated cotton cloth and the chitosan film sample and it also softened the film sample, making it supple rather than the rigid film that it was before autoclaving.

Chitosan Gel

Chitosan samples were prepared by dissolving 1g, 0.1g, 0.01g or 0.001g (+/– 0.0001g) of chitosan in 10 mls acetic acid (2M, pH5). The 1g sample was so thick it needed heating to 70 ºC to fully dissolve.

Chitosan Suspension

To 1g, 0.1g or 0.01g chitosan was added to 10mls of distilled water. The chitosan did not dissolve and thus needed constant agitation to keep the chitosan powder in suspension.

Chitosan Film

Chitosan was dissolved in acetic acid (2M, pH5) and the acid was allowed to evaporate, leaving a film of chitosan (and traces of un-evaporated acetic acid). No attempt was made to remove acetic acid residues. For materials coated in chitosan, materials were dipped in 0.1% (w/v) chitosan in acetic acid solution and then allowed to hang dry in a fume cupboard for 12 hrs.

Chitosan Coated Cotton cloth

The chitosan-coated cotton cloth was made by dipping woven cotton cloth (made at Heriot-Watt University) first in chitosan solution (0.1g chitosan dissolved in 100mls acetic acid (2M, pH5)) and then transferred into a NaOH bath (0.1M pH 13 in excess) to neutralise the acid and precipitate the chitosan and then the excess chitosan was squeezed out of the material using a glass rod on a glass plate. The samples were then washed under cold water and hung on an aluminium bar to dry at ~20°C (room temperature) for 24hrs.

The control was cotton cloth treated in acetic acid without the chitosan and neutralised in sodium hydroxide and washed in water then dried in the same way.

Chitosan Fibre

Attempts were made to try to produce useable chitosan fibres but these were not entirely successful, although this could yield more success with a suitable investment of time. Initial attempts yielded some success but within the project there was neither the time nor more importantly, the equipment available to yield useful results.

1g of chitosan was added to 20mls dilute acetic acid (2M, pH5) and mixed using a glass rod. This was then left for half an hour to dissolve. The resulting thick gel was then filtered through a Buchner funnel and extruded using a syringe with a 1ml pipette tip attached into a 2M NaOH bath. The fibre was then collected from the NaOH bath and dried on a glass rod.

Culture Media

Standard Nutrient Agar (NA) plates

This process was scaled to make the required quantity of NA plates. To make 5 NA plates (containing approx 20mls of agar each), 2.8g NA powder and 100mls distilled water were measured out. The NA powder was added to the distilled water in a glass bottle and swirled to mix. A cap was placed on the bottle (loosely, to prevent the bottle exploding inside the autoclave) and autoclaved in a Dixons Vario 2228 autoclave at 121°C for 15 minutes. When the autoclaved solution was cool enough to handle, the solution was removed from the autoclave and Swirled until no concentration haze was observable at the bottom of the bottle. The mixture was then allowed to cool to ~ 60ºC. When the solution had cooled, approx 20mls of NA solution was poured onto each Petri dish (10 cm) and then allowed to set. The NA plates were then left for 24 hours at around 20ºC before use to remove excess moisture. As a rule, more NA plates were produced than were required to allow for unforeseen circumstances.

Chitosan NA plates

This process was scaled to make the required quantity of chitosan NA plates. To make 3 NA plates containing each acetic acid solution (containing approx 20mls of agar each), 2.8g NA powder and 90mls distilled water were measured out into 5 different bottles. 5 bottles of 10 mls acetic acid were prepared with varying quantities of chitosan powder added to each of the 5 bottles (1g, 0.1g, 0.1g, 0.001g chitosan or no chitosan for the control). The 5 bottles were swirled to mix. A cap was placed on each bottle (loosely, to prevent the bottle exploding inside the autoclave) and autoclaved in a Dixons Vario 2228 autoclave at 121°C for 15 minutes. When the autoclaved solutions were cool enough to handle, the solutions were removed from the autoclave and Swirled until no concentration haze was observable at the bottom of the bottle. The mixtures were then allowed to cool to ~ 60ºC. When the solutions had cooled, approx 20mls of each solution was poured onto 3 Petri dishes and then allowed to set. The plates were then left for 24 hours at around 20ºC before use to remove excess moisture.

Standard Nutrient Broth (NB)

This process was scaled to make the required quantity of NB. To make 10 bottles of NB (containing 10mls of NB each), 2.5g NB powder and 100mls distilled water were measured out. The NB powder was added to the distilled water in a glass bottle and swirled to mix. A cap was placed on the bottle (loosely, to prevent the bottle exploding inside the autoclave) and autoclaved in a Dixons Vario 2228 autoclave at 121°C for 15 minutes. When the autoclaved solution was cool enough to handle, the solution was removed from the autoclave and Swirled until no concentration haze was observable at the bottom of the bottle. The mixture was then allowed to cool to ~ 60ºC. When the solution had cooled, 10mls of NB solution was dispensed into 10 sterilised 25ml Culture bottles (universal bottles or universals). The NB bottles were then allowed to cool to room temperature (20ºC).

Chitosan NB

This process was scaled to make the required quantity of chitosan NB. To make 3 NB universals containing each acetic acid solution (containing approx 10mls of broth each), 5g NB powder and 200mls distilled water were added to a bottle. The bottle was swirled to mix. 5 bottles of 10 mls of distilled water were prepared with varying quantities of chitosan powder added to each (1g, 0.1g, 0.1g, 0.001g chitosan or no chitosan for the control). A cap was placed on each bottle (the NB solution, the chitosan suspensions, the control and 15 universals) with the caps attached loosely (to prevent the bottles exploding inside the autoclave) and autoclaved in a Dixons Vario 2228 autoclave at 121°C for 15 minutes. When the autoclaved solutions were cool enough to handle, the solutions were removed from the autoclave and the NB solution was swirled until no concentration haze was observable at the bottom. The NB solution, the chitosan suspensions and the 15 universals were then allowed to cool to ~ 60ºC. When the solutions had cooled, 9mls of NB solution was dispensed into each of the 15 universals. 1 ml of each chitosan suspension was added to 3 universals (vortexing the suspensions prior to extracting the suspension using a vortex mixer). 1ml of distilled water was added to the 3 remaining universals (vortexing the water prior to extracting the suspension for consistency). The NB mixtures were allowed to cool to room temperature (20ºC) prior to use.

Chitosan Film Plates

As for standard NA plates but with chitosan film added to plate after inoculation with bacteria. Any air pockets under chitosan film were squeezed out.

Chitosan Coated Material Plates

As for chitosan film plates, but with the chitosan film having a material embedded (cotton cloth or polypropylene mesh).

3.1.2 Methods

Examination of Chitosan as a Bacteriostat

Methodology

The experimental methods for the chitosan study were derived from discussions with academic staff after an extensive review of the available literature. The methods were designed primarily to examine the bacteriostatic effect of chitosan in relation to hospital pathogens and evolved into a study that examined how the quantity of chitosan available and the form in which the chitosan was presented affected the bacteria.

Cell Count

Using the Gilson 20µl pipette, take 10µl of cells. Stain cells using methylene blue (10µl methylene blue to 10µl cell suspension). Place methylene blue stained cells on Haemocytometer (improved Neubauer BS748, depth 0.01mm, 1/400mm^2) and place cover slip on top of the drop of cells. Place Haemocytometer on microscope. Count cells in 10 random squares. Cells are counted when in the middle of the square (not touching the lines) and when in contact with the bottom and left sides of the square. Cells touching the top and left sides are excluded from the cell count figure. Get the average of the 10 cell counts. Divide the average by 16, then multiply by 4. multiply that figure by 10^6 and you have the cells per ml.

Method 1 - Chitosan dissolved in dilute acetic acid incorporated into nutrient agar Vs MRSA 9551 and Staphylococcus epidermis

This experiment was designed to examine the growth of MRSA 9551 and Staphylococcus *epidermis* on nutrient agar plates containing chitosan gel.

Obtain cultures of MRSA 9551 and Staphylococcus *epidermis* and culture the Staphylococcus *epidermis* on nutrient agar and the MRSA 9551 on DST agar for 24 hrs. Dissolve 1g chitosan in 10mls acetic acid (2M). Dissolve 0.1g chitosan in 10mls acetic acid (2M). Add 1g chitosan in 10mls acetic acid (2M) to 90mls nutrient agar. Add 0.1g chitosan in 10mls acetic acid (2M) to 90mls nutrient agar. Add 10mls acetic acid (2M) to 90mls nutrient agar. Prepare 100 ml nutrient agar. Prepare 2x 10mls 0.9% saline solution. Autoclave the prepared nutrient agars and saline solutions at 121ºC for 15 minutes. Shake (swirl) autoclaved agars well without producing bubbles, allow to cool to 40-50ºC and pour into Petri dishes (approximately 20mls each) and allow to cool to room temperature.

Take the 24 hour culture of MRSA and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Take the 24 hour culture of MRSA and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Perform cell count of the saline inoculums using Neubauer improved haemocytometer. Add 100mls of MRSA inoculum to the control (NA), the control containing acetic acid (NA + acetic acid), the NA + 1g chitosan in 10 ml acetic acid, NA + 0.1g chitosan in 10 ml acetic acid and spread the inoculum across the plates with sterile glass beads. Add 100mls of Staphylococcus *epidermis* inoculum to the control (NA), the control containing acetic acid (NA + acetic acid), the NA + 1g chitosan in 10 ml acetic acid, NA + 0.1g chitosan in 10 ml acetic acid and spread the inoculum across the plates with sterile glass beads. Inoculate 2 NA plates, one with MRSA and the other with Staphylococcus *epidermis* and spread the inoculum across the plate with sterile glass beads, then add a 1cm square of chitosan film to each. Incubate at 37°C for 48hrs and then examine for signs of growth.

Method 1b - Modified method

This method is a modified version of method 1. By neutralising the acetic acid control and using only the 1g chitosan and 0.1g chitosan samples, all of the agar plates would be solid enough to inoculate. In addition, the plates were inoculated using a sterile swab of saline inoculum instead of an inoculation loop (to increase the quantity of inoculum).

Obtain cultures of MRSA 9551 and Staphylococcus *epidermis* and culture Staphylococcus *epidermis* on nutrient agar and MRSA 9551 on DST agar for 24 hrs. Dissolve 1g chitosan in 10mls acetic acid (2M). Dissolve 0.1g chitosan in 10mls acetic acid (2M). Add 1g chitosan in 10mls acetic acid (2M) to 90mls nutrient agar. Add 0.1g chitosan in 10mls acetic acid (2M) to 90mls nutrient agar. Add 10mls acetic acid (2M) (neutralised to pH 7 using NaOH) to 90mls nutrient agar. Prepare 100 ml nutrient agar. Autoclave the prepared nutrient agars and saline solutions at 121ºC for 15 minutes. Shake (swirl) autoclaved agars well without producing bubbles, allow to cool to 40-50ºC and pour into Petri dishes (approximately 20mls each) and allow to cool to room temperature.

Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate the control (NA) making sure to cover the entire plate. Repeat this process for the control containing acetic acid (NA + acetic acid), the NA + 1g chitosan in 10 ml acetic acid, NA + 0.1g chitosan in 10 ml acetic acid. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of Staphylococcus *epidermis* and inoculate the control (NA) making sure to cover the entire plate. repeat this process for the control containing acetic acid (NA + acetic acid), the NA + 1g chitosan in 10 ml acetic acid, NA + 0.1g chitosan in 10 ml acetic acid. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the entire plate and then add a 1cm square of chitosan film. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the entire plate and then add a 1cm square of chitosan film. Incubate at 37°C for 48hrs and then examine for signs of growth.

Method 2 - Chitosan dissolved in acetic acid added to nutrient broth Vs MRSA 9551 and Staphylococcus epidermis

Method 2 was redesigned so that the experiment would be performed using nutrient broth and measuring the growth of the bacteria spectrophotometrically using a LKB Biochrom Ultrospec II. With this study, all of the samples and the control contained acetic acid.

Obtain cultures of MRSA 9551 and Staphylococcus *epidermis* and culture on Staphylococcus *epidermis* nutrient agar and MRSA 9551 on DST agar for 24 hrs. Add 1g chitosan to 10mls acetic acid (2M). Add 0.1g chitosan to 10mls acetic acid (2M). Add 0.01g chitosan to 10mls acetic acid (2M). Make 100 ml nutrient broth (2.5g nutrient broth powder + 100mls distilled water). Make 4 x 110 ml chitosan nutrient broth (2.5g nutrient broth powder + 100mls distilled water) +; (1g chitosan + 10 ml acetic acid (2M)), (0.1g chitosan + 10 ml acetic acid (2M)), (0.01g chitosan + 10 ml acetic acid (2M)) and (10 ml acetic acid (2M)). Autoclave the prepared nutrient broth and 0.9% saline solution and 10 glass culture bottles (~25ml) at 121ºC for 15 minutes. Shake (swirl) autoclaved broth well and allow to cool to room temperature. Dispense 2 (x10mls) of each media into a universal (2x NB, 2x NB +10mls acetic acid (2M), 2x NB +10mls acetic acid (2M) + 1g chitosan, 2x NB +10mls acetic acid (2M) + 0.1g chitosan and 2x NB +10mls acetic acid (2M)+ 0.01g chitosan).

Take the 24 hour culture of MRSA and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Take the 24 hour culture of Staphylococcus *epidermis* and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Perform cell count of the saline inoculums using Neubauer improved haemocytometer. Add 0.5ml of MRSA to one of each of the nutrient broth. Add 0.5mls of Staphylococcus *epidermis* one of each of the nutrient broth. Incubate at 37°C. After 2 hours take 1 ml of bacterial broth from each culture and add each sample to a 1ml spectrophotometry curvette. Measure the absorbance of the samples

at AD550nm. Examine every 2 hours for 8 hours using the spectrophotometer and then once after 24 hours.

Method 2b - Modified method - Chitosan added to nutrient broth Vs MRSA 9551 and Staphylococcus epidermis

In this method, the chitosan powder was not dissolved in acetic acid. Instead, it was suspended in distilled water. This was to study how colloidal chitosan affected bacterial growth and to remove any effect the pH may have on bacterial growth.

Obtain cultures of MRSA 9551 and Staphylococcus *epidermis* and culture on Staphylococcus *epidermis* nutrient agar and MRSA 9551 on DST agar for 24 hrs. Prepare 1g chitosan in 10mls distilled water. Prepare 0.1g chitosan in10mls distilled water. Prepare 0.01g chitosan in 10mls distilled water. Make 5 x 110 ml nutrient (2.5g nutrient broth powder + 100mls distilled water +; (1g chitosan + 10mls distilled water), (0.1g chitosan + 10mls distilled water), (0.01g chitosan + 10mls distilled water) and Prepare 100 ml nutrient broth. Autoclave the prepared nutrient broth and 0.9% saline solution and 12 glass culture bottles (~25ml) at 121ºC for 15 minutes. Shake (swirl) autoclaved broth well and allow to cool to room temperature. Dispense 3 (x10mls) of each media into a universal vortexing each time to resuspend chitosan (3x NB, 3x NB + 1g chitosan, 3x NB + 0.1g chitosan and 3x NB + 0.01g chitosan).

Take the 24 hour culture of MRSA and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Take the 24 hour culture of Staphylococcus *epidermis* and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Perform cell count of the saline inoculums using Neubauer improved haemocytometer. Add 0.5ml of MRSA to one of each of the nutrient broth. Add 0.5mls of Staphylococcus *epidermis* one of each of the nutrient broth. Incubate all of the samples (including the sterile controls) at 37°C and examine every hour for 4 hours using spectrophotometer at AD550nm and then once after 24 hours.

Method 2c - Modified method - Chitosan added to nutrient broth Vs MRSA 9551 and Staphylococcus epidermis

This method is a further refinement of method 2b. In method 2b the nutrient broths were stationary when in the incubator. This method includes the use of a platform shaker to encourage the chitosan powder to remain in suspension while in the incubator. The platform shaker agitated the chitosan powder into suspension therefore it was necessary to let the chitosan powder to settle before spectrophotometer readings to prevent the chitosan suspension from influencing the absorbance readings. An absorption wavelength of 550nm was used for the spectrophotometer as is it the optimal wavelength for bacterial turbidity readings.

Obtain cultures of MRSA 9551 and Staphylococcus *epidermis* and culture on Staphylococcus *epidermis* nutrient agar and MRSA 9551 on DST agar for 24 hrs. Prepare 1g chitosan in 10mls distilled water. Prepare 0.1g chitosan in10mls distilled water. Prepare 0.01g chitosan in 10mls distilled water. Prepare 5 x 110ml nutrient (2.5g nutrient broth powder + 100mls distilled water +; (1g chitosan + 10mls distilled water), (0.1g chitosan + 10mls distilled water), (0.01g chitosan + 10mls distilled water) and Prepare 100ml nutrient broth. Autoclave the prepared nutrient broth and 0.9% saline solution and 8 glass culture bottles (~25ml) at 121ºC for 15 minutes. Shake (swirl) autoclaved broth well allow to cool to room temperature. Dispense 3 (x10mls) of each media into a universal (2x NB, 2x NB + 1g chitosan, 2x NB + 0.1g chitosan and 2x NB + 0.01g chitosan).

Take the 24 hour culture of MRSA and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Take the 24 hour culture of Staphylococcus *epidermis* and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Add 0.2ml of MRSA to one of each of the nutrient broth. Add 0.2mls of Staphylococcus *epidermis* one of each of the nutrient broth. Incubate all of the samples (including the sterile controls) at 37°C on a platform shaker and examine every hour for 4 hours using spectrophotometer at AD550nm and then once after 24

hours (allow the chitosan suspension to settle ~15mins to before taking spectrophotometer readings).

Method 3 - Testing of Chitosan treatment of Cotton cloth

This experiment was designed to examine the efficacy of chitosan coatings on a material (cotton cloth) to inhibit bacterial growth of MRSA 9551 and Staphylococcus *epidermis*. This method is a development of the chitosan film sample tested in method 1.

Obtain cultures of MRSA 9551 and Staphylococcus *epidermis* and culture Staphylococcus *epidermis* and MRSA 9551 in nutrient broth for 24 hrs. Prepare 120 ml nutrient agar and 60mls 0.9% saline solution. Autoclave the prepared nutrient agars, saline solution and cotton cloth samples at 121ºC for 15 minutes. Shake (swirl) autoclaved agars well without producing bubbles, allow to cool to 40-50ºC and pour into 6 Petri dishes (approximately 20mls each) then allow to cool to room temperature.

Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the entire plate. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the entire plate, then add a 1cm square of untreated cotton cloth. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the entire plate, then add a 1cm square of chitosan coated cotton cloth. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the entire plate. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of Staphylococcus *epidermis* and inoculate a nutrient agar making sure to cover the entire plate, then add a 1cm square of untreated cotton cloth. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of Staphylococcus *epidermis* and inoculate a nutrient agar making sure to cover the entire plate, then add a 1cm square of chitosan

coated cotton cloth. Incubate at 25°C for 72 hrs and then examine for signs of growth.

Method 3b - Modified method - Testing of Chitosan treatment of Cotton cloth

This method is a refined version of method 3. The samples were covered in aluminium foil to maintain the sterility while cooling down from the autoclave cycle and instead of using a swab to inoculate the agar plates, 20 µl of inoculum was used to standardise the quantity of bacteria on each agar plate.

Obtain cultures of MRSA 9551 and Staphylococcus *epidermis* and culture Staphylococcus *epidermis* and MRSA 9551 in nutrient broth for 24 hrs. Prepare 120 ml nutrient agar. Autoclave the prepared nutrient agars, saline solution and cotton cloth samples at 121ºC for 15 minutes. Cotton cloth samples were wrapped in aluminium foil during the autoclave cycle. Shake (swirl) autoclaved agars well without producing bubbles, allow to cool to 40-50ºC and pour into 6 Petri dishes (approximately 20mls each) and allow to cool to room temperature.

Dispense 20µl of MRSA broth onto a nutrient agar and spread around the NA using a Bunsen sterilised inoculation loop making sure to cover the entire plate, then add a 1cm square of untreated cotton cloth using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Dispense 20µl of MRSA broth onto a nutrient agar and spread around the NA using a Bunsen sterilised inoculation loop making sure to cover the entire plate, then add a 1cm square of chitosan coated cotton cloth using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Dispense 20µl of Staphylococcus *epidermis* broth onto a nutrient agar and spread around the NA using a Bunsen sterilised inoculation loop making sure to cover the entire plate, then add a 1cm square of untreated cotton cloth using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Dispense 20µl of Staphylococcus *epidermis* broth onto a nutrient agar and spread around the NA using a Bunsen sterilised inoculation loop making sure to cover the entire plate, then add a 1cm square of chitosan coated cotton cloth using Bunsen sterilised tweezers and

squeeze out any air bubbles under the samples. Incubate at 25°C for 72 hrs and then examine for signs of growth.

Method 3c - Modified method - Testing of Chitosan treatment of Cotton cloth & polypropylene

This method is a modified version of method 3. The method is the same as for method 3 with the addition of a chitosan coated polypropylene mesh. In addition, the samples were placed in glass bottles (with lids) to prevent the moisture in the autoclave from effecting the chitosan coating and to maintain the sample sterility until they were used.

Obtain cultures of MRSA 9551 and Staphylococcus *epidermis* and culture Staphylococcus *epidermis* and MRSA 9551 in nutrient broth for 24 hrs. Prepare 200 ml nutrient agar and 100mls 0.9% saline solution. Autoclave the prepared nutrient agars, saline solution and cotton cloth samples at 121ºC for 15 minutes. Cotton cloth and polypropylene samples were placed in "universal" bottles during the autoclave cycle to keep them dry (as the samples were dry after the autoclave, they would need moistening with 0.9% saline so they would adhere to the agar). Shake (swirl) autoclaved agars well without producing bubbles, allow to cool to 40-50ºC and pour into 6 Petri dishes (approximately 20mls each) and allow to cool to room temperature.

Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the entire plate, then add a 2cm square of untreated cotton cloth (sterilised) using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the entire plate, then add a 2cm square of chitosan coated cotton cloth (sterilised) using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the

entire plate, then add a 2cm square of chitosan coated cotton cloth (non-sterilised) using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of MRSA and inoculate a nutrient agar making sure to cover the entire plate, then add a 2cm square of chitosan coated polypropylene mesh (sterilised) using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples.

Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of Staphylococcus *epidermis* and inoculate a nutrient agar making sure to cover the entire plate, then add a 2cm square of untreated cotton cloth (sterilised) using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of Staphylococcus *epidermis* and inoculate a nutrient agar making sure to cover the entire plate, then add a 2cm square of chitosan coated cotton cloth (sterilised) using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of Staphylococcus *epidermis* and inoculate a nutrient agar making sure to cover the entire plate, then add a 2cm square of chitosan coated cotton cloth (non-sterilised) using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Take a sterile swab and dip it in sterile saline solution (0.9%) then take a swab of Staphylococcus *epidermis* and inoculate a nutrient agar making sure to cover the entire plate, then add a 2cm square of chitosan coated polypropylene mesh (sterilised) using Bunsen sterilised tweezers and squeeze out any air bubbles under the samples. Incubate at 25°C for 72 hrs and then examine for signs of growth.

<u>Method 4 - Chitosan suspended in nutrient broth Vs MRSA</u>

Prepare 3 litres of plate count agar and 2 litres of 0.9% saline solution. Sterilise the plate count agar and 0.9% saline solution in the autoclave at 121ºC for 15 minutes. Shake (swirl) autoclaved agars well without producing bubbles, allow to cool to 40-50ºC and pour into and poured into 200 Petri dishes (approximately 20mls each) and allow to cool to room temperature. Once the plate count agars have cooled, store for 1 week to dry out a little (so that when they are inoculated, there isn't excess

moisture enabling the bacteria to spread). Obtain culture of MRSA 9551 and culture on nutrient agar for 24 hrs. Accurately weigh out 1g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Accurately weigh out 0.8g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Accurately weigh out 0.6g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Accurately weigh out 0.4g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Accurately weigh out 0.2g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Sterilise 200 'Universals'. Dispense 9mls of 0.9% saline solution into 150 'universals'. Dispense 10 ml of distilled water in to a "universal" bottle (25ml) (the control). Prepare 100mls of nutrient broth. Prepare 3x 10mls 0.9% saline solution in "universal" bottles (25ml). Sterilise the chitosan samples, nutrient broths and saline solution in saline in the autoclave at 121ºC for 15 minutes. Take 1ml of the chitosan/ distilled water mixture (mix by pipetting 3x first) and add to 9mls of nutrient broth).

Take the 24 hour culture of MRSA and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Perform cell count of the saline inoculum using Neubauer improved haemocytometer. Add 200mls of MRSA inoculum to the control (NB + 1ml distilled water), the NB + 1g chitosan, NB + 0.1g chitosan, NB + 0.01g chitosan. Incubate at 37°C for 48hrs on a platform shaker and then examine for signs of growth. Take 1ml of each sample and add to 9mls 0.9% saline, vortex mix, then take 1ml of the inoculated saline and inoculate it into 9mls 0.9% saline. Repeat a further 5 times for 10^{-6} dilution and 7 times for 10^{-8} dilution. The 48hr samples should be diluted to 10^{-8} and 10^{-6}, 10^{-7} & 10^{-8} samples should be used to inoculate plate count agars. (100ul per plate count agar, spread across the plate count agar using sterile glass beads). Return cultures to platform shaker in 37ºC incubator after the dilutions have been performed. The 72hr Samples should be taken and diluted to 10^{-8} and 10^{-6}, 10^{-7} & 10^{-8} samples should be used to inoculate plate count agars. (100ul per plate count agar, spread across the plate count agar using sterile glass beads). Return cultures to platform shaker in 37ºC incubator after the dilutions have been performed. The plate count agars had the colonies counted 24 - 48 hours after inoculation and the results were noted.

Method 4b - Modified method

This method is a refined version of method 4. The concentrations of chitosan (and the control) are performed in triplicate (e.g. control 1, control 2 and control 3). In addition, the dilutions performed have been expanded to 10^{-9} on certain days in order to have plate count agars containing countable numbers of colonies.

Prepare 4 litres of plate count agar. Sterilise the plate count agar in the autoclave at 121ºC for 15 minutes. Shake (swirl) autoclaved agars well without producing bubbles, allow to cool to 40-50ºC and pour into and poured into 200 Petri dishes (approximately 20mls each) and allow to cool to room temperature. Once the plate count agars have cooled, store for 1 week to dry out a little (so that when they are inoculated, there isn't excess moisture enabling the bacteria to spread). Obtain culture of MRSA 9551 and culture on nutrient agar for 24 hrs. Accurately weigh out 1g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Accurately weigh out 0.8g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Accurately weigh out 0.6g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Accurately weigh out 0.4g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Accurately weigh out 0.2g chitosan and add to 10 ml of distilled water in to a "universal" bottle (25ml). Dispense 10 ml of distilled water in to a "universal" bottle (25ml) (the control). Sterilise 200 'Universals' and prepare 2 litres of sterile 0.9% saline solution. Dispense 9mls of 0.9% saline solution into 150 'universals'. Prepare 100mls of nutrient broth. Prepare 3x 10mls 0.9% saline solution in "universal" bottles (25ml). Sterilise the chitosan samples, nutrient broths and saline solutions in the autoclave at 121ºC for 15 minutes. Take 1ml of the chitosan/ distilled water mixture (mix by pipetting 3x first) and add to 9mls of nutrient broth).

Take the 24 hour culture of MRSA and inoculate an inoculation loop full of MRSA into 10mls 0.9% saline and use a vortex mixer to ensure through mixing. Add 100mls of MRSA inoculum to the control (NB + 1ml distilled water), the NB + 0.1g chitosan, NB + 0.08g chitosan, NB + 0.06g chitosan, NB + 0.04g chitosan, NB + 0.02g chitosan (Perform this stage in triplicate). Perform cell count of the saline

inoculum using Neubauer improved haemocytometer. Incubate at 37°C for 48hrs on a platform shaker and then examine for signs of growth.

Dilution procedure;

take 1ml of each sample and add to 9mls 0.9% saline, vortex mix, then take 1ml of the inoculated saline and inoculate it into 9mls 0.9% saline. Repeat a further 5 times for 10^{-6} dilution and a further 7 times for 10^{-9} dilution, ensuring to vortex mix each dilution.

Samples should be taken and diluted to 10-8 after 48 hours and 10^{-6} - 10^{-8} samples used to inoculate plate count agars. (100ul per plate count agar, spread across the plate count agar using sterile glass beads). Once dilutions are performed, wash the universals and repeat step 11 and 12 so that the salines are ready for the next day. Samples should be taken and diluted to 10-9 after 72 hrs and 10^{-7} - 10^{-9} samples used to inoculate plate count agars (100ul per plate count agar, spread across the plate count agar using sterile glass beads). Once dilutions are performed, wash the universals and repeat step 11 and 12 so that the salines are ready for the next day. Samples should be taken and diluted to 10-8 after 96 hours and 10^{-6} - 10^{-8} samples should be used to inoculate plate count agars. (100ul per plate count agar, spread across the plate count agar using sterile glass beads). The plate count agars should have the colonies counted ~48 hours after inoculation and the results should be noted.

3.2 Production of Biomaterial Samples

Material / Details	Poly-ε-Caprolactone 6400	Solanyl	Polylactic acid	Polyester	Polypropylene	Tuftane Polyurethane
Source	Solvay	Rodenburg Biopolymers	Cargill Dow	Vascutek	Borealis polypropylene	Lord Corporation
Grade	6400	Flexibilitis	N/A	VP1200K Virgin grade	N/A	N/A
Method of fabrication	Extrusion	Extrusion	Film casting - dissolved in dichloromethane (DCM)	Extrusion followed by knitting	Extrusion	extrusion
Melting Point (°C)	62.5	112.5	168	257.5	151	149.5
Extrusion Temperature (°C)	76	125 @280psi	N/A	N/A	235	N/A
Tape / sample width (mm)	1.33	0.97	1.13	2	1.4	1
Tape / sample thickness (mm)	0.09	0.19	0.01	0.9	0.16	0.05
Additional notes	Hand drawn over 47°C roller		Unable to extrude a useful tape, therefore prepared as a film	Obtained in the form of pre fabricated vascular graft		Obtained as a pre fabricated sheet

Table 3.1 Sample summary.

Table 3.1 illustrates the source and production methods used to produce the samples used in the experiments.

3.2.1 Extrusion

Polypropylene tape

Materials

Polypropylene pellets MFI-19 (borealis polypropylene)

ESL vertical extruder (model 250)

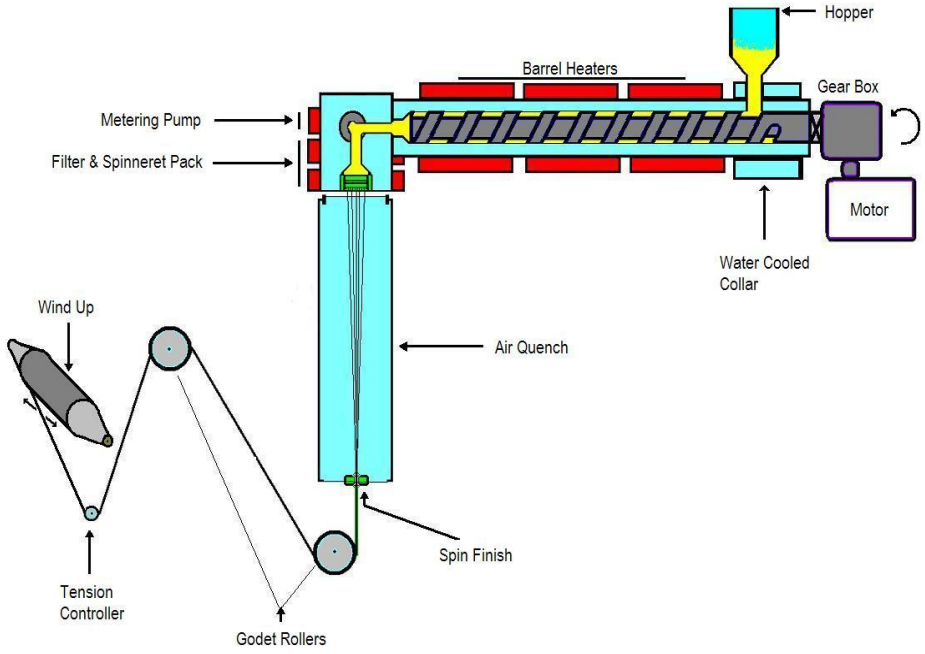


Fig 3.2 (Younes et al., 2009) - Diagram of ESL vertical extruder illustrating the extruder screw, die head (in green), the air quench chamber and winding apparatus. The barrel heaters are divided into zones so that the temperature of the molten polymer can be controlled from where it enters the extruder screw through to the die head. The extruder screw forces the polymer through the barrel, increasing the pressure of the molten polymer until it reaches the die head.

Method

The polypropylene tape was produced with the following extruder settings.

Extruder		
Zone 1	Zone 2	Zone 3
180°C	180°C	185°C
Pump	Die Head	
193°C	Zone 1	Zone 2
	208°C	211°C
Melt	Extruder screw speed	
212°C	19.6-18.7 rpm	
Pre pump pressure	Die Head Pressure	
769-860psi	514psi	
Metering Pump	Air Quench	Winder
4.1rpm	23%	3rpm
Polymer Draw Frame		
Roller No1	Roller No2	Roller No4
34mpm	80mpm	158mpm
80ºC	80ºC	80ºC

Table 3.3 polypropylene extrusion parameters. These setting were determined by Stewart Wallace, the extrusion technician at Heriot-Watt University.

Solanyl

Materials

Solanyl Flexibilitis pellets

ESL Laboratory Extrusion, Melt Spinning and Draw Equipment. Labspin 892

Fig 3.4 ESL Laboratory Extrusion, Melt Spinning and Draw Equipment. Labspin 892.

Method

The Solanyl tape was produced with the following extruder settings

Material Details	
Source	Rodenburg Biopolymers
Grade	Flexibilitis
Method of fabrication	Extrusion
Melting Point (°C)	119.2
Extrusion Temperature (°C)	125 @280psi
Tape / sample width (mm)	0.97
Tape / sample thickness (mm)	0.19

Table 3.5 Solanyl extrusion parameters. These setting were determined by Stewart Wallace, the extrusion technician at Heriot-Watt University.

Solanyl + 2% Chitosan Powder (W/W)

This was extruded as per Solanyl but was mixed with chitosan powder at 2% w/w prior to extrusion.

Poly-ε-caprolactone 6400 tape

Materials

Solvay poly-ε-caprolactone

Bradford University Research Ltd. Small Scale Ram Extruder

Fig. 3.6 Bradford University Research Ltd. Small Scale Ram Extruder

Method

The poly-ε-caprolactone tape was produced with the following extruder settings.

<table>
<tr><td colspan="3">Extruder</td></tr>
<tr><td>Zone 1</td><td>Zone 2</td><td>Zone 3</td></tr>
<tr><td>80°C</td><td>80°C</td><td>80°C</td></tr>
<tr><td>Pump</td><td colspan="2">Die Head</td></tr>
<tr><td rowspan="2">105°C</td><td>Zone 1</td><td>Zone 2</td></tr>
<tr><td>105°C</td><td>105°C</td></tr>
<tr><td>Melt</td><td>Extruder screw speed</td><td rowspan="4"></td></tr>
<tr><td>105°C</td><td>19.6-18.7 rpm</td></tr>
<tr><td>Pre pump pressure</td><td>Die Head Pressure</td></tr>
<tr><td>769-860psi</td><td>514psi</td></tr>
<tr><td>Metering Pump</td><td>Quench Tank</td><td>Winder</td></tr>
<tr><td>1.5rpm</td><td>10.8%</td><td>3rpm</td></tr>
</table>

Table 3.7 poly-ε-caprolactone extrusion parameters. These setting were determined by Stewart Wallace, the extrusion technician at Heriot-Watt University.

3.2.2 Film Casting

PLA film

Perform all work using Dichloromethane in a fume cupboard. 1g of PLA (Cargill Dow) fibre is placed in a 200ml Pyrex glass beaker. Add 30mls Dichloromethane (DCM) (Acros Organics). Wait for the PLA to dissolve completely. Pour solution on glass sheet and place in rack for glass plates. Wait for the DCM to evaporate (takes about 4 hours but can be left longer). Collect the film. Place the film in an airtight bag and squeeze out any air and store it at room temperature in the bag until required.

3.2.3 Plasma Treatment

Materials

Polypropylene

Tuftane polyurethane

Polyester (Vascutek polyester VP1200K™)

Poly-ε-Caprolactone 6400 tape

Solanyl

PLA

Plasma treatment at Riccarton campus (Nanotech)

Equipment

Argon gas

Ammonia gas

Pirani 10 Pressure gauge

Thruline Watt meter (model 43, Biro Electronic Corporation, Cleveland Ohio)

Parallel plate plasma equipment (pressure chamber parallel plates and purge system by Nanotech, model PE250, serial 115)

Vacuum pump

RF generator (solid state power generator, Eni Powersystems Inc, model OEM-6, serial 729)

Fume cupboard (to vent the spent gases)

Silane calibrated flow meter to be used for argon gas (therefore actual gas flow rate = output reading x [flow factor for new gas/flow factor for the calibrated gas] = output reading x 1.4 [1.4 is the argon conversion factor] /0.4 [0.4 is the silane conversion factor])

Ammonia calibrated flow meter

Method

Recommended settings

Pressure 10^{-1}Torr

Power 50-100W

Zero flow (for silane calibrated flow meter) registers as 0.5cc (therefore all flow readings will be compensated for by removing the 0.5cc

Recommended gas flow (valves open) is 20cc

Electrode gap 2.5cm

Safety checks

Check the cooling water for the RF unit is running. Check the RF power is off when the chamber is open. For Argon treatment - Set the regulator on the gas cylinder to a maximum of 5 bar.

Procedure

Before first run (warm up)

Before any treatment takes place, the following need to be performed to prepare the equipment (argon gas is the vent/purge gas)

Close the plasma chamber and turn on the vacuum pump. Flush the system with argon gas to purge out any other gases (open the needle valve and turn on the electric valve). Set flow meter to 20 cubic centimetres (cc). Adjust the pressure to recommended levels (10^{-1}Torr). Turn on RF and tune for 0 reflected power (by adjusting input and load controls) and record forward power. Turn power off. Turn gas off. Vent gas.

For Argon treatment

Fume cupboard should be checked to make sure it is on before anything else to vent any waste gases. Open argon cylinder (5 bar max). Turn on vacuum pump. Purge gas lines and plasma chamber with Argon. Turn on water-cooling for RF generator. Turn on the rest of the equipment (gauges, valves). Perform dummy run to ensure RF generator and gas flow are set to desired specifications. Pump out the chamber to about 10^{-1} Torr (open the valve to the pump) and periodically vent the chamber with argon (will partially release the vacuum) and repeat at least 5 times to ensure air has been removed (displaced by the argon). Pump down chamber for trial treatment to 10^{-1} Torr (no sample). Adjust gas flow (for treatment gas) until the pressure within the chamber is 20^{-1} Torr and record the gas flow. Turn on the RF generator and adjust the power to desired level. Check the Watt meter and adjust settings until there is 0 reflected power (all the power is going forward). Equipment should be set now for your samples so, close the valve to the pump and fill the chamber with argon to return the pressure to atmospheric pressure. Place samples on lower plate. Pump down chamber for treatment to 10^{-1} Torr. Open the treatment gas valve (the flow rate is already set). When ready, turn on the RF generator (power level already set) and administer RF power for a measured time (for the treatment used, the time is 1 minute). When time has expired, turn off the RF generator. Vent the chamber to atmospheric pressure (close the pump valve and admit argon to the chamber to relieve the vacuum).

For Ammonia gas treatment

Prior to commencing the ammonia gas line needs to be vented with argon (as the gas line is shared with other gases) the rest of the procedure is the same as for argon, except for the addition of step 15.

For potentially toxic or malodorous treatment gases, add more vent/ pump down cycles after step 14 to remove treatment gas completely from the chamber prior to relieving the pressure to atmospheric pressure and opening the treatment chamber.

Flow rates (excess gas used for both gases)

Argon mean flow rate = 28.35cc

Ammonia mean flow rate = 9.8cc

(Operating pressures were the same = 20^{-1} Torr)

RF time = 1minute

Fig. 3.8

Nanotech plasma chamber

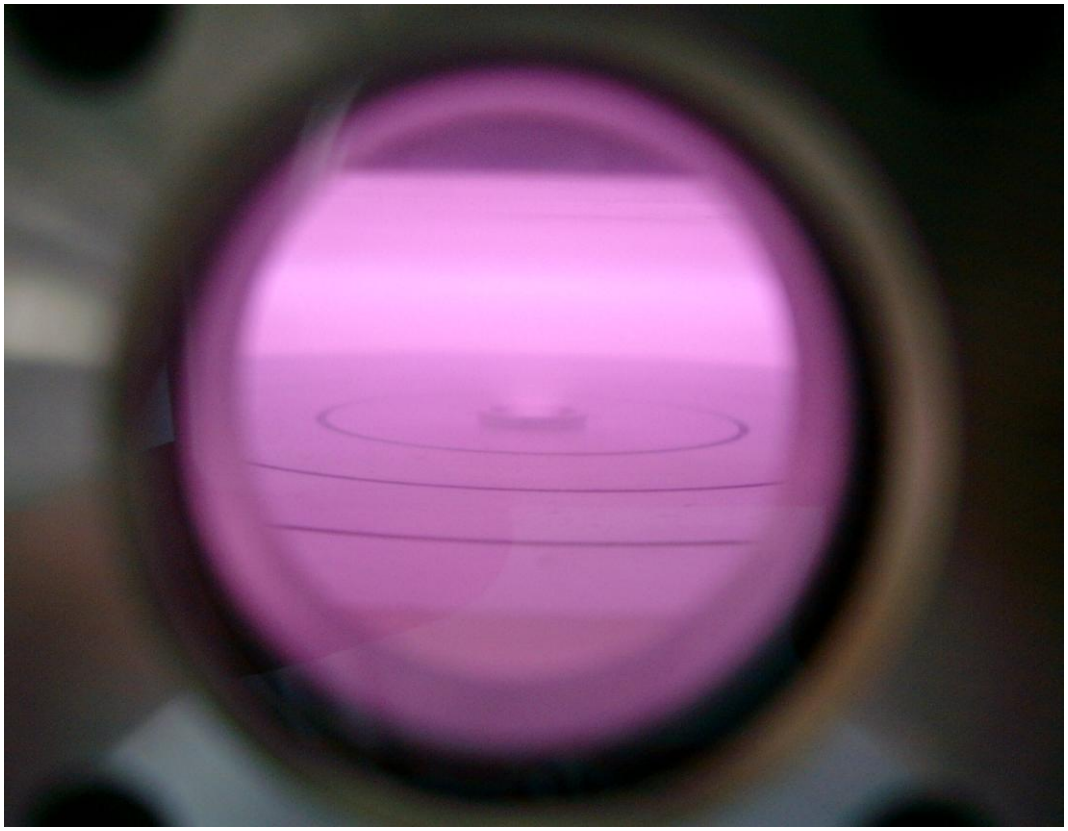


Fig. 3.9

View of the plasma chamber during warm up showing the high energy plasma

Europlasma Plasma Treatment

Equipment

Argon Gas

Oxygen Gas

Europlasma Surface Treatment CD400PC MHz System

The following settings were used (settings were stored as file **mike2**)

Gas Flow	0.4 SLM (standard Litres per Minute)
Power	300W
RF Time	5Mins
Pressure	200Mtorr

Method

Place sample to be treated in the plasma chamber. Load configuration file "**mike2**" and allow the process to run. Collect and store sample in an airtight bag at room temperature.

Fig. 3.10

Europlasma plasma treatment machine showing the computerised controls on the left hand side and the plasma chamber on the right hand side

3.3 Sample Characterisation

3.3.1 Differential scanning calorimetry (DSC) Analysis

All standard materials (untreated) were analysed by DSC (Mettler DSC 12E). This was done to determine the melting point. Samples were placed in aluminium crucibles and heated. The temperature increased at 5ºC per minute.

3.3.2 SEM Analysis

The electron microscope was used to examine the standard materials and plasma treated materials to determine if there was any observable physical change to the material surface due to plasma treatment.

The materials first needed to be splutter coated for 60 seconds using a Polaron sc7620 splutter coater before being examined in a Hitachi S-530 scanning electron microscope.

Method

Instrument Switch On

Turn on the cooling water about 2 full turns (tap marked blue). Switch on the power at the wall (LOW, *WARM UP* and STOP lamps will glow red). Move (lower) EVAC POWER lever to on position (up). Press the EVAC button on console (LOW and WARM UP lamps will glow red). Wait for 20 minutes until HIGH lamp is lit green.

Sample Preparation

Samples are prepared by placing them on SEM stubs (1cm aluminium disks with a female thread on their base corresponding to the SEM sample mount) in the Polaron splutter coater to coat them with a fine film of platinum, so the microscope can see the surface.

Fig. 3.11 - Polaron sc7620 splutter coater

Fig. 3.12 - Hitachi S-530 scanning electron microscope

Introducing Samples to Column

Press AIR button. Wait until hear an audible hiss. Open the sliding drawer. Screw the sample stub on. Close the sliding drawer and hold. Press EVAC button (pump will kick in). Wait (around 2 minutes) until HIGH lamp is lit green.

Image Formation

Move (lower) DISPLAY lever to on position (up). Wait until ACC VOLTAGE READY lamp is lit steady red (not flashing). Switch on ACC VOLTAGE (normally 5 or 10 kV). Press the left-most SCANNING SPEED button (TV rate, 0). FOCUS control: switch to AUTO and press COARSE button to produce image. Flick WFM switch (under concealing panel) down. Adjust FILAMENT knob clockwise (to about 2 o'clock position) until trace at maximum height position on screen [if necessary use MANUAL CONTRAST BRIGHTNESS to make trace visible on screen). Press the left-most SCANNING SPEED button to restore image. Press ABC button twice under AUTO condition to optimise brightness and contrast. To suit eye, B and C can be controlled by switching to MANUAL and rotating lower B and C knobs. Use AUTO (coarse / fine) or MANUAL control to adjust image focus. Move around sample at low magnification to locate position of interest. Adjust magnification to required level, focusing as required for image quality.

Instrument Shutdown

Reduce magnification to lowest. Turn ACC VOLTAGE off. Wait for about 1 minute, then move (lower) DISPLAY lever to off position (down). Press AIR button, await audible hiss. Remove sample. Close drawer, press EVAC button, wait until HIGH lit green. Depress STOP button and wait until LOW and STOP lamps lit red. Move (lower) EVAC POWER lever to off position (down). Wait for around 20 minutes. Switch off instrument at wall. Turn off cooling water.

Analysis of Pore Size of PLA Sample

The PLA pore size was determined by selecting SEM image representative of the PLA SEM images and measuring the dimensions of each pore (the horizontal and vertical), measuring the area of the pores using a ruler and calculating the percentage of pores in relation to the area of the image.

3.4 Tissue Culture Study

3.4.1 Methodology

This experimental method was derived after reading through research papers and observing a gap in the research. Many papers extolled the benefits of a particular material or examined explanted devices from human or animal subjects. The primary aim of this study was to conduct a basic study to evaluate a range of materials on a quantitative level. In addition to the standard materials, modified materials were included so that the modifications could be evaluated directly with the standard materials. This study was designed to be as simple and as controlled as possible. Capillary tubes were used to act as ballast to prevent the samples from floating.

Background

This experiment was designed to evaluate a range of materials for their ability to support human cell growth. This was a simple experiment that used MRC-5 cells to determine which material / surface treatment was optimal. Initially, Human foetal fibroblasts were going to be used but the cells from the supplier were at the end of their passage limit and subsequently died very quickly. The cells were seeded directly onto the test material with no additional materials used to encourage attachment (e.g. Matrigel). Gelatine was tested as a means to improve cell attachment but it was discarded as it would influence the results.

Preparation

The samples needed mounting for the tissue culture study so that the samples would sink when placed in the tissue culture media. Glass capillary tubes were chosen as they would provide the necessary ballast to ensure the samples remained submerged. The samples were then sterilised at Anderson Caledonia using ethylene gas. Ethylene gas was chosen as it did not involve high temperatures that could melt some of the polymers with low melting points.

Samples Preparation

Materials

10 cm soda glass capillary tubes

Glass cutter

Paperclip

70% Ethanol

Sterilisation Bags

Samples

Polypropylene

Argon plasma treated polypropylene (Nanotech)

Ammonia plasma treated polypropylene (Nanotech)

Polypropylene coated in chitosan

Argon plasma treated polypropylene coated in chitosan (Nanotech)

Ammonia plasma treated polypropylene coated in chitosan (Nanotech)

Tuftane polyurethane

Argon plasma treated Tuftane polyurethane (Nanotech)

Ammonia plasma treated Tuftane polyurethane (Nanotech)

Argon plasma treated Tuftane polyurethane coated in chitosan (Nanotech)

Argon Plasma treated Tuftane polyurethane (Europlasma)

Oxygen plasma Treated Tuftane polyurethane (Europlasma)

Vascutek polyester

Argon plasma treated Vascutek polyester (Nanotech)

Ammonia plasma treated Vascutek polyester (Nanotech)

Poly lactic acid

Argon plasma treated poly lactic acid (Nanotech)

Ammonia plasma treated poly lactic acid (Nanotech)

Poly-ε-Caprolactone 6400

Argon plasma treated poly-ε-caprolactone 6400 (Nanotech)

Ammonia plasma treated poly-ε-caprolactone 6400 (Nanotech)

Solanyl

Argon plasma treated Solanyl (Nanotech)

Ammonia plasma treated Solanyl (Nanotech)

Solanyl extruded with 2% chitosan (w/w)

Solanyl coated in chitosan

Argon plasma treated Solanyl coated in chitosan (Nanotech)

Ammonia plasma treated Solanyl coated in chitosan (Nanotech)

Method

Cut capillary tubes into 3cm lengths using a glass cutter. Cut samples into 4cm lengths. Insert into the capillary tubes using a paperclip to poke the ends in. The samples were prepared in excess (19 of each sample + one un-mounted for analysis in SEM). Rinse samples 5 times with 70% ethanol and then placed in gas sterilisation bags. Sterilise samples at Anderson Caledonia (ethylene gas sterilised).

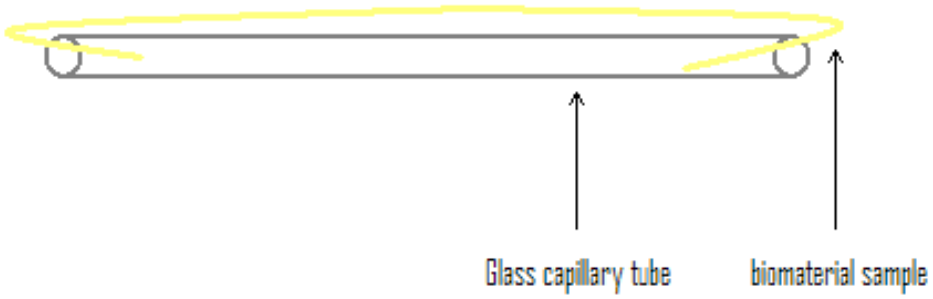


Fig 3.13 - Demonstration of how the biomaterial sample was mounted to the capillary tube.

3.4.2 Experimental Work

For this experiment, the samples were seeded with a small drop of MRC-5 cells and the samples were then inoculated over 29 days.

Materials

Trypsin (10x Concentration) 100ml. Invitrogen

Fetal Bovine Serum, certified (heat inactivated) Origin U.S. Invitrogen

Performance, mycoplasma, virus bacteriophage and endotoxin tested

Culture Medium - McCoy's 5a + 2mM Glutamine

Gilson Pipettes - P20, p200, p1000 and p10 ml Pipettes

Phosphate Buffered Saline Tablets

$25cm^3$ Iwaki® Culture Flasks (Non-Treated, Hydrophobic surface)

Centrifuge

Tissue Culture Incubator (37ºC, 5%CO_2)

-80˚C freezer

Media (modified minimal essential eagles medium) = 500mls minimal essential eagles medium + 50ml FBS + 11ml l-glutamine +5.5 ml NEAA

L-lysine

NEAA (nonessential amino acids)

DMSO (Dimethyl sulfoxide**)**

Trypsin

FBS (Fetal bovine serum)

PBS (Phosphate buffered saline)

Flasks

Cryogenic storage tubes

2um filters

Centrifuge tubes

Pipettes & tips

Water bath

LaminAir hood

Biocide ZF (Spray Disinfectant for Incubators and Sterile Cabinets in Cell Culture Area)

Ethanol

10% Chloros

Haemocytometer- improved Neubauer BS748, depth 0.01mm, 1/400mm^2

Samples

Sterile tweezers

Iwaki® 25ml tissue culture flasks (both treated and untreated)

Liquid N_2

Liquid N_2 Storage

Centrifuge

Water bath

Molecular Probes "live or dead" viability/cytotoxicity kit (L-3224) (Invitrogen)

- Contains Calcein AM and Ethidium Homodimer-1

Human Foetal Lung Fibroblasts (http://www.ecacc.org.uk/)

Cell Line Name	MRC-5
ECACC No.	97112601
Cell Line Description	Established from normal lung tissue of a 14 week old male foetus. The cells undergo between 60-70 population doublings before senescence. The virus susceptibility of this line is similar to WI-38. This cell line is supplied on a standing order basis.
Species	Human
Tissue	Lung, foetal
Morphology	Fibroblast
Sub Culture Routine	Split sub-confluent cultures (70-80%) 1:3 to 1:6 i.e. seeding at 2-4 x 10,000 cells/cm using 0.25% Trypsin or Trypsin/EDTA; CO2; 37C.
Culture Medium	EMEM (EBSS) + 2mM Glutamine + 1% Non Essential Amino Acids (NEAA) + 10% Foetal Bovine Serum (FBS).
Karyotype	2n = 46, diploid
Depositor	Dr P Jacobs, NIBSC, London
Country	UK
References	(Jacobs et al., 1970)

Table 3.14 Summary of MRC-5 cells

Method

Preparation

To ensure the experiment would not have any glitches, the planning stage was vital to make the experiment as controlled as possible. The most important factor was to ensure the cells were all in the same condition / passage number and consumables were available when required.

Standard methods

Preparation for any work in the LaminAir hood (Heraeus HS12)

Turn on hood 30mins prior to work to stabilize air flow. Clean the LaminAir hood with Biocide ZF. Clean the LaminAir hood with Ethanol. Then sterilise the LaminAir hood with UV. Clean everything with Ethanol before placing in the LaminAir hood.

Procedure for Thawing Cells

Warm up media and place in suitably labelled tissue culture flask. Remove the chosen cells from the liquid nitrogen storage. Place the cryo vial in the 37ºC water bath for ~ 1minute. Before the pellet is completely thawed, remove the vial, clean the vial (with biocide ZF) and pace in the LaminAir hood. Immediately empty the contents of the vial into 1ml of the pre-warmed media. Place the media and cells in a centrifuge tube and down at 2000rpm for 4mins at 30°C (Heraeus Megafuge 1.0R). Pour off the media and Re-suspend the cell pellet in 2mls of fresh media. Empty the re-suspended cells in a tissue culture flask containing 8mls of pre-warmed media. Attach the flask cap loosely and place in the incubator.

Passage procedure

Warm up media, Trypsin and PBS (no Ca^{2+} or Mg^{2+}) to 37°C for ~30mins before use in a Grant OLS200 water bath. Examine cells carefully. If cells are ~70% confluent, then proceed with passage. If there is contamination, dispose of the cells. If the cells are less than ~70% confluent but the media has turned orange/yellow, change media. If passage is required, dispose of old media. Use PBS (no Ca^{2+} or Mg^{2+}) to wash cells once (use pipette (~10mls) then dispose of PBS. Add 2mls of Trypsin and place in incubator for 2mins at 37°C. Once cells detach (Trypsin = orange), give the flask a tap against the side of a hard object to dislodge the cells from the bottom of the flask. Check cells on the microscope (Axiovert 25). They should be rounded and floating freely in the media. If any cells remain attached to the bottom of the flask, give the flask an additional tap. Add 2mls media (10% FBS) to neutralize the Trypsin. Put in centrifuge tube and spin down at 2000rpm for 4mins at 30°C. Dispose of media + Trypsin. Add 8mls media. Re-suspend cell pellet in new flask. Check cells under inverted microscope. Place cells in incubator (37ºC, 5% CO_2).

Cell count procedure

Dispose of old media. Use PBS (no Ca^{2+} or Mg^{2+}) to wash cells once (use pipette (~10mls) then dispose of PBS. Add 2mls of Trypsin and place in incubator for 2mins at 37°C. Once cells detach (Trypsin = orange), give the flask a tap against the side of

a hard object to dislodge the cells from the bottom of the flask. Check cells. They should be rounded and floating freely in the media. If any cells remain attached to the bottom of the flask, give the flask an additional tap. Add 2mls media (10% FBS) to neutralize the Trypsin. Take 10µl of cells. Stain cells using methylene blue (10ul methylene blue to 10ul cell suspension). Place methylene blue stained cells on Haemocytometer (improved Neubauer BS748, depth 0.01mm, 1/400mm^2) and place cover slip on top of the drop of cells. Place Haemocytometer on microscope (Zeiss Axiovert 25). Count cells in 10 random squares. Cells are counted when in the middle of the square (not touching the lines) and when in contact with the bottom and left sides of the square. Cells touching the top and left sides are excluded from the cell count figure. Obtain the average of the 10 cell counts. Divide the average by 16 and then multiply by 4. Multiply that figure by 10^6 and you have the cells per ml.

Procedure for Freezing Cells (cryogenic storage)

Take cells after step 9 of passage procedure and wash the cells with media (containing FBS). Centrifuge cells again as per step 9 of passage procedure. Re-suspend cells in freezing medium (10% DMSO, 20% FBS and 70% standard media). DMSO is filter sterilized using a 2um filter. Dispense cells into cryo tubes. When freezing, do it slowly (1hr @ 4°C, 1hr @ -20°C and 1hr @ -80°C then place in liquid nitrogen).

Procedure for fluorescence staining of the samples

Remove the LIVE/DEAD reagent stock solutions from the freezer and allow them to warm to room temperature. Add 20µL of the supplied 2mM EthD-1 stock solution to 10ml of sterile, tissue culture–grade D-PBS, vortexing to ensure thorough mixing. This gives an approximately 4µM EthD-1 solution. Combine the reagents by transferring 5µL of the supplied 4 mM calcein AM stock solution (Component A) to the 10mL EthD-1 solution. Vortex the resulting solution to ensure thorough mixing. The resulting approximately 2µM calcein AM and 4µM EthD-1 working solution is then added directly to cells. Note that aqueous solutions of calcein AM are susceptible to hydrolysis. Aqueous working solutions should therefore be used

within one day. Cut the sample off the capillary tube mounting and place sample in a Petri dish. Add 100–150µl of the combined LIVE/DEAD assay reagents, using optimized concentrations, to the surface of the sample. Incubate the cells for 30–45 minutes at room temperature. Following incubation, add about 10µL of the fresh LIVE/DEAD reagent solution or D-PBS to a clean microscope slide. Using fine-tipped forceps, carefully (but quickly) invert and mount the sample on the microscope slide. Place the slide on the on the Leica DMIRE2 confocal microscope. Set the microscope to 500nm for the Calcein stain and 550nm for the ethidium stain. View the labelled samples under the fluorescence microscope.

Experimental Technique

Before the experiment could commence, the cells from the ECACC needed to be grown to sufficient quantities to supply the entire experiment. To do this, the cells were initially split into 4 flasks (1-4) and then frozen. Each batch were then grown and split and the passages were recorded as n, n.x, n.x.y, n.x.y.z so the vials could be easily traced back to the original split. This also made it easier to ensure the cells used for the experiment were all from the same passage stage. It was essential to ensure the cells in the experiment were from the same passage number, as non-immortalised cells in culture only have a finite number of passages before they die and the cells health varies with passage level. This was one level of continuity built into the experiment.

Before the start of each run, the cells to be used were resuscitated from cryogenic storage and given time to recover and reach ~ 70% confluences. The cells were then Trypsinised, centrifuged and re-suspended in 1ml of media.

The samples were inoculated with 20µl of cell suspension and left for ~10 minutes in an empty flask before media was added, to give the cells a chance to attach to the substrate without the media washing them off. After the 10mins, 10mls of media was added and the samples were placed in the incubator (37□C 5% CO_2) with loosened caps.

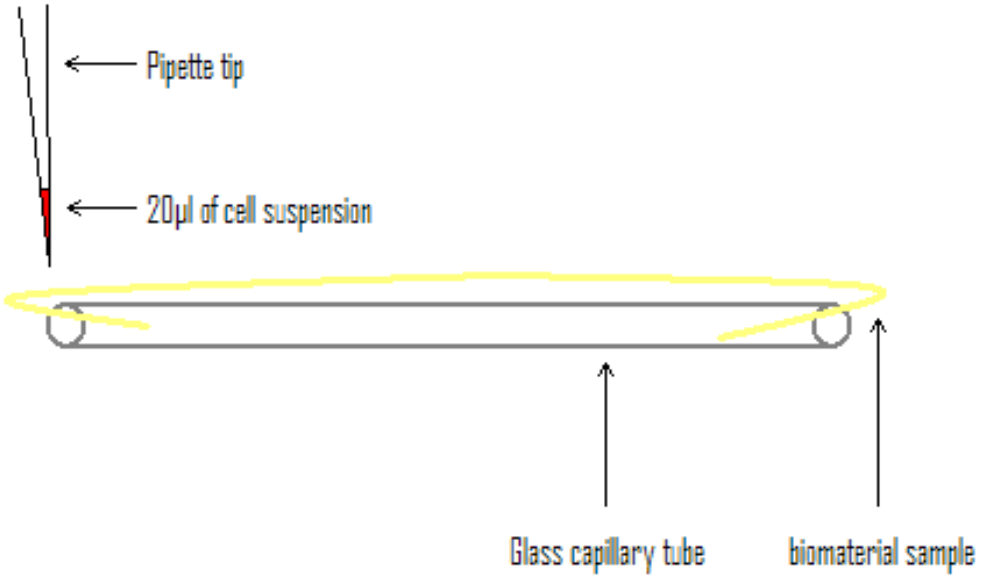


Fig. 3.15 - Illustration of biomaterial inoculation.

After inoculation, a cell count was performed. Sample groups were started in three groups per month, two days apart. Growth along the sample was measured using a photocopy of a ruler (Helix shatter proof) with millimetre markings on acetate after 7 days, 14 days 21 days and 29 days. The same acetate ruler was used throughout the experiment (the acetate copy was compared to the original ruler to ensure the gradations were accurate). At the end of the 29 day study period, the samples were removed from the capillary tube mounting and placed in Petri dishes. The samples were then stained with Molecular Probes "live or dead" viability/cytotoxicity kit (L-3224) and examined on the Leica DMIRE2 confocal microscope to confirm the level of growth.

Chapter 4 - Results

4.1 Examination of Chitosan as a Bacteriostat

The chitosan study was devised to test the reported antimicrobial effects of chitosan. The experiments were designed to present chitosan to MRSA and Staphylococcus *epidermis* and examine how effective chitosan is against the opportunistic pathogens. In addition, examining how the bacteriostatic effect varied with the quantity of chitosan presented to the bacteria would clarify how varying the chitosan quantity would alter growth. Through the development of the experimental design, the experiment evolved. Performing studies where the chitosan was presented to the bacteria in different forms, while not directly comparable with each other added an interesting dimension to the study.

Method 1 - Chitosan dissolved in dilute acetic acid incorporated into nutrient agar Vs MRSA 9551 and Staphylococcus epidermis.

Method 1 was the preliminary study designed to evaluate the efficacy of chitosan as a bacteriostat.

Many of the chitosan (+ acetic acid) plates did not solidify enough to inoculate. This included the acetic acid control, the 0.001g & 0.01g chitosan samples and some of the 0.1g & 1g chitosan samples. This was due to the acetic acid hydrolysing the agar and destroying the structure of the polysaccharide.

Cell counts	MRSA 9551	*S. epidermis*
1	54	7
2	41	12
3	51	7
4	43	6
5	48	8
6	44	21
7	36	15
8	42	10
9	46	21
10	37	9
Average	44.2	11.6

Table 4.1 - Cell count data for MRSA and S. *epidermis* inoculum - haemocytometer count (volume of square = 1/400ml)

Therefore to reach bacteria per ml;

((Average cells per box) x 4) x (10^{-6}) = Cells per ml

(44.2 x 4) x 10^{-6} = 1.77 x 10^{9} bacteria per ml for MRSA

(11.6 x 4) x 10^{-6} = 4.64 x 10^{8} bacteria per ml for S. *epidermis*

	MRSA 9551	S. *epidermis*
Control 1 (NA)	1 small cream colony (less than 1mm)	5 small yellow colonies (less than 1mm)
Control 2 (NA)	1 small yellow colony (less than 1mm) not cream (like MRSA) therefore contamination	1 small orange colony (under agar) not cream (like MRSA) therefore contamination
NA + Chitosan Film	29 small colonies less than 0.5mm in diameter, all in one location around initial streak. No growth anywhere near the film	No colonies
NA + 1g Chitosan in 10 ml acetic acid (1)	No growth	No growth
NA + 1g Chitosan in 10 ml acetic acid (2)	No growth	No growth
NA + 1g Chitosan in 10 ml acetic acid (3)	No growth	No growth
NA + 0.1g Chitosan in 10 ml acetic acid	No growth	No growth

Table 4.2 - Results after 48 hrs

In this experiment, growth was low and the control containing acetic acid did not solidify due to hydrolysis of the agar therefore this experiment was revised.

Method 1b - Modified method – Chitosan dissolved in dilute acetic acid incorporated into nutrient agar Vs MRSA 9551 and Staphylococcus epidermis

Method 1b is a modified version of method 1.

The NA control was kept and instead of the NA + acetic acid control, NA + acetic acid neutralised to pH 7 (using NaOH and a corning pH meter 215) was used. The NA + 1g chitosan in 10 ml acetic acid and NA + 0.1g chitosan in 10 ml acetic acid agar plates were re-used after re-sterilisation (as there was no previous growth).

In addition, instead of diluting the bacteria, each plate was inoculated from the saline inoculum with a sterile swab.

The chitosan film was also included in this experiment. The NA plates with the chitosan film were inoculated before adding the film, so the growth could be examined to see if the bacteria would grow up to, under or over the film.

After inoculation, the plates were incubated for 48hrs at 37°C

	MRSA 9551	S. epidermis
NA control	Good growth. A lawn grew from where the plate was inoculated. No contamination.	Good growth. A lawn grew from where the plate was inoculated. No contamination.
NA + neutralised acetic acid control	Good growth. A lawn grew from where the plate was inoculated. No contamination.	Good growth. A lawn grew from where the plate was inoculated. No contamination.
Chitosan film on NA	Bacterial growth surrounding film. No growth on film.	Bacterial growth surrounding film. No growth on film.
1g Chitosan + 1ml acetic acid in NA	No growth, bacteria still present. The plate appears no different from when inoculated. (Bacteriostatic effect)	No growth, bacteria still present. The plate appears no different from when inoculated. (Bacteriostatic effect)
0.1g Chitosan + 1ml acetic acid in NA	No growth, bacteria still present. (Bacteriostatic effect)	No growth, bacteria still present. (Bacteriostatic effect)

Table 4.3 - Results after 48 hrs

This experiment produced interesting data for how the bacteria reacted in the presence of chitosan film, but the data for the nutrient agar containing chitosan was less clear. The acetic acid control still did not provide a suitable control for the chitosan samples (as the chitosan could not be neutralised without the chitosan precipitating and not mixing with the agar).

With this in mind, Nutrient broth appeared to be a better choice than nutrient agar as this would avoid the problem of agar hydrolysis.

Method 2 - Chitosan dissolved in acetic acid added to nutrient broth Vs MRSA 9551 and Staphylococcus epidermis

Three quantities of chitosan powder were weighed out; 1g, 0.1g and 0.01g (+ /- 0.001g). These samples were added to 10 ml acetic acid (2M), forming a range of solutions designed to avoid the problem of agar hydrolysis by substituting nutrient agar for nutrient broth. This method uses spectrophotometry to measure the turbidity (cloudiness resulting from the bacterial growth) to produce quantitative data. The samples were inoculated using a standard inoculum (an inoculation loop of bacteria mixed in a saline solution, and then counted).

Cell counts	MRSA 9551	S. epidermis
1	34	20
2	12	25
3	36	30
4	38	18
5	38	33
6	54	22
7	44	34
8	40	41
9	45	30
10	57	32
Mean	39.8	28.5

Table 4.4 - Cell count data for MRSA and S. *epidermis* inoculum.

Haemocytometer Vol = 1/400 ml per small square (visible through microscope)

Therefore for;

MRSA = $(39.8 * 4) * (10^6) = 1.59 \times 10^9$ bacteria per ml

S. *epidermis* = $(28.5 * 4) * (10^6) = 1.14 \times 10^9$ bacteria per ml

The broths were then inoculated with 0.5mls of the bacterial dilutions (either MRSA 9551 or S. epidermis).

Cell density was measured spectrophotometrically for Staphylococcal species using a wavelength of 550nm. Samples were measured every 2 hrs to examine for changes in growth. After 2hrs there was growth in the NB control, but there was no growth in the acetic acid control. The pH was inhibiting bacterial growth. The experimental design would have to change so that the pH was no longer an issue.

Method 2b - Modified method - Chitosan added to nutrient broth Vs MRSA 9551 and Staphylococcus epidermis

This experiment was redesigned so the chitosan would not be dissolved into solution. This would determine if chitosan in suspension would elicit the desired bacteriostatic effect. Three quantities of chitosan powder were weighed out; 1g, 0.1g and 0.01g (+ /- 0.001g). These samples were added to 10 ml distilled water, forming a range of suspensions (the powder did not remain in suspension long before settling out and therefore needed constant agitation).

1ml of the suspension was added to each of the chitosan nutrient broths (therefore the 1g becomes 0.1g, although it is still referred to as 1g) and 1ml of distilled water was added to the control.

The samples were inoculated directly from an inoculation loop to increase the quantity of bacteria present in each broth. After inoculation, the samples were placed on a shelf in a 37ºC incubator.

Sample	Contents	AD550nm (11.30 - 11.45am) (+/- 0.007)
Control (ref)	NB	0.001
Control 0.01g	NB + 1ml (0.01g chitosan /10mls distilled water)	0.007
Control 0.1g	NB + 1ml (0.1g chitosan /10mls distilled water)	-0.005
Control 1g	NB + 1ml (1g chitosan /10mls distilled water)	0.065
S. *epidermis* Control	NB + 0.5mls S. epidermis broth	-0.005
MRSA Control	NB + 0.5mls MRSA 9551 broth	-0.005
S. *epidermis* 0.01g	NB + 1ml (0.01g chitosan /10mls distilled water) + 0.5mls S. epidermis broth	-0.004
S. *epidermis* 0.1g	NB + 1ml (0.1g chitosan /10mls distilled water) + 0.5mls S. epidermis broth	0.007
S. epidermis 1g	NB + 1ml (1g chitosan /10mls distilled water) + 0.5mls S. epidermis broth	0.077
MRSA 0.01g	NB + 1ml (0.01g chitosan /10mls distilled water) + 0.5mls MRSA 9551 broth	-0.006
MRSA 0.1g	NB + 1ml (0.1g chitosan /10mls distilled water) + 0.5mls MRSA 9551 broth	-0.004
MRSA 1g	NB + 1ml (1g chitosan /10mls distilled water) + 0.5mls MRSA 9551 broth	0.089

Table 4.5 - Absorbance reading at Time 0

Sample	AD550nm
Control (ref)	0.000
Control 0.01g	-0.004
Control 0.1g	0.002
Control 1g	0.007
S. *epidermis* Control	-0.005
MRSA Control	-0.007
S. *epidermis* 0.01g	-0.004
S. *epidermis* 0.1g	0.011
S. epidermis 1g	-0.004
MRSA 0.01g	-0.005
MRSA 0.1g	-0.002
MRSA 1g	0.025

Table 4.6 - Absorbance reading at 1hr

Sample	AD550nm
Control (ref)	0.006 reset to 0.000
Control 0.01g	-0.004
Control 0.1g	-0.003
Control 1g	0.001
S. *epidermis* Control	-0.002
MRSA Control	-0.010
S. *epidermis* 0.01g	-0.004
S. *epidermis* 0.1g	0.004
S. epidermis 1g	0.130
MRSA 0.01g	-0.015
MRSA 0.1g	-0.008
MRSA 1g	0.102

Table 4.7 - Absorbance reading at 2hrs

3 hrs

This experiment was abandoned as there was no growth in the controls.

Method 2c - Modified method - Chitosan added to nutrient broth Vs MRSA 9551 and Staphylococcus epidermis

This is a modified version of method 2b. In this method, the nutrient broths were kept on a platform shaker to keep the chitosan powder in suspension.

Sample Name	Description
Reference	Control (NB)
S. epidermis	NB + 0.5mls S. epidermis broth
S. *epidermis* 0.01g	NB + 1ml(0.01g chitosan /10mls distilled water) + 0.5mls S. epidermis broth
S. *epidermis* 0.1g	NB + 1ml(0.1g chitosan /10mls distilled water) + 0.5mls S. epidermis broth
S. epidermis 1g	NB + 1ml(1g chitosan /10mls distilled water) + 0.5mls S. epidermis broth
MRSA	NB + 0.5mls MRSA 9551 broth
MRSA 0.01g	NB + 1ml(0.01g chitosan /10mls distilled water) + 0.5mls MRSA 9551 broth
MRSA 0.1g	NB + 1ml(0.1g chitosan /10mls distilled water) + 0.5mls MRSA 9551 broth
MRSA 1g	NB + 1ml(1g chitosan /10mls distilled water) + 0.5mls MRSA 9551 broth

Table 4.8 – Sample descriptions.

Time 0

Samples inoculated and absorbance measured (AD550nm).

The samples were left for 15mins to allow the chitosan suspension to settle before taking spectrophotometer readings.

Reference = NB.

Sample	AD550nm
Reference	-0.001 (+/- 0.005)
S. epidermis	0.000 (+/- 0.005)
S. *epidermis* 0.01g	-0.010 (+/- 0.004)
S. *epidermis* 0.1g	0.000 (+/- 0.004)
S. epidermis 1g	0.135 (+/- 0.004)
MRSA	-0.010 (+/- 0.001)
MRSA 0.01g	-0.016 (+/- 0.002)
MRSA 0.1g	-0.004 (+/- 0.003)
MRSA 1g	0.113

Table 4.9 - Absorbance reading at Time 0

Sample	AD550nm
Reference	0.000 (+/- 0.004)
S. epidermis	0.000 (+/- 0.004)
S. *epidermis* 0.01g	-0.001 (+/- 0.003)
S. *epidermis* 0.1g	0.000 (+/- 0.002)
S. epidermis 1g	0.113
MRSA	0.006 (+/- 0.002)
MRSA 0.01g	-0.006 (+/- 0.004)
MRSA 0.1g	0.001 (+/- 0.003)
MRSA 1g	0.125

Table 4.10 - Absorbance reading at 1hr

Sample	AD550nm
Reference	0.000 (+/- 0.001)
S. epidermis	0.005 (+/- 0.002)
S. *epidermis* 0.01g	0.003 (+/- 0.001)
S. *epidermis* 0.1g	0.006 (+/- 0.002)
S. epidermis 1g	0.070 (+/- 0.001)
MRSA	0.016 (+/- 0.004)
MRSA 0.01g	0.005 (+/- 0.001)
MRSA 0.1g	0.007 (+/- 0.001)
MRSA 1g	0.080 (+/- 0.003)

Table 4.11 - Absorbance reading at 2hrs

Sample	AD550nm
Reference	0.000 (+/- 0.001)
S. epidermis	0.020 (+/- 0.002)
S. *epidermis* 0.01g	0.008 (+/- 0.001)
S. *epidermis* 0.1g	0.008 (+/- 0.001)
S. epidermis 1g	0.017 (+/- 0.002)
MRSA	0.160 (+/- 0.002)
MRSA 0.01g	0.148 (+/- 0.002)
MRSA 0.1g	0.147
MRSA 1g	0.174 (+/- 0.002)

Table 4.12 - Absorbance reading at 3hrs

After 25 hrs the samples were taken out of the 37°C incubator and shaken to re-suspend the bacteria and chitosan. In the 1g chitosan and S. *epidermis* sample, It was noticed the chitosan powder (which settles quite quickly (~5-10 minutes) was looking bigger (particle size). When it settled, it was less dense and of greater volume. It appears to have agglutinated with the S. *epidermis*. With this observation, it was decided to examine the other samples closely. It appeared that it had the same effect on the other S. *epidermis* samples, but not with the MRSA 9551 samples. This was an unexpected outcome as S. *epidermis* and MRSA 9551 are closely related. It was decided to continue with taking the spectrophotometer readings and see what differences that revealed.

Sample	AD550nm
Reference	0.000 (+/- 0.001)
S. epidermis	0.450 (+/- 0.001)
S. *epidermis* 0.01g	0.502 (+/- 0.001)
S. *epidermis* 0.1g	0.429 (+/- 0.001)
S. epidermis 1g	0.113 (+/- 0.002)
MRSA	0.558
MRSA 0.01g	0.510 (+/- 0.001)
MRSA 0.1g	0.466 (+/- 0.002)
MRSA 1g	0.549 (+/- 0.001)

Table 4.13 - Absorbance reading at 25hrs

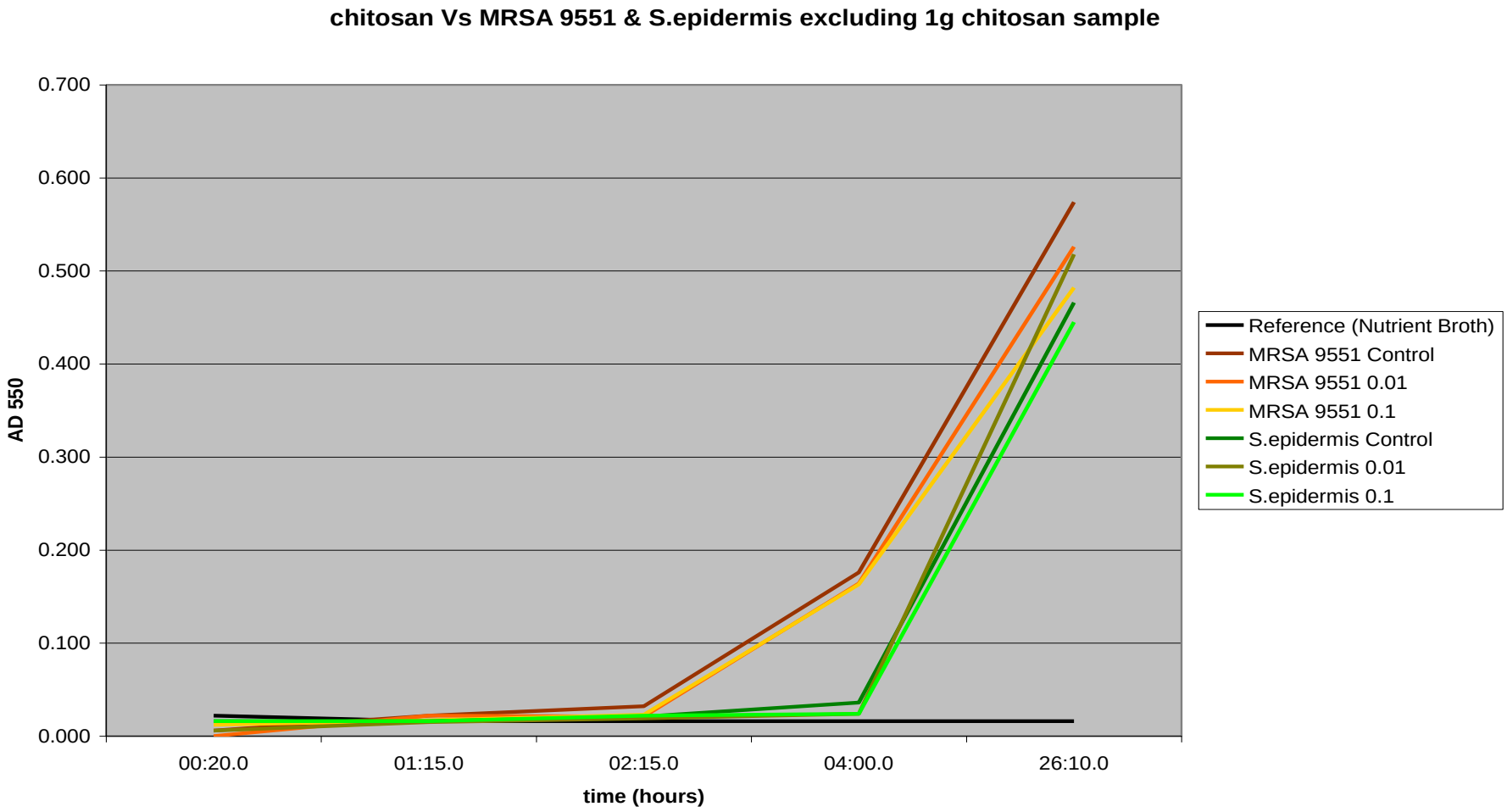


Fig. 4.14 - Absorbance readings for MRSA and S. *epidermis*

Fig. 4.14 has the values for NB + 1ml (1g chitosan/10mls distilled water), NB + 1ml (1g chitosan/10mls distilled water) + *S. epidermis* and the NB + 1ml (1g chitosan/10mls distilled water) + MRSA removed. This was because the high level of chitosan was distorting the absorbance readings. The original graph is in appendix A.2, Method 2c. This graph shows two results. The chitosan appears to have a bacteriostatic effect on MRSA as shown by the reduced growth of the samples with 0.01g of chitosan and 0.1g of chitosan. This bacteriostatic effect also appears to be related to the quantity of chitosan present. The S. *epidermis* does not appear to demonstrate reduced growth in the presence of chitosan.

Method 3 - Testing of Chitosan treatment of Cotton cloth

For this study, two types of cotton cloth were used, chitosan gel coated and untreated. Hypothesis; inoculate NA plate so that a bacterial lawn will develop and place cotton cloth on top (treated or untreated) and observe for signs of inhibition around edge of material.

After 24 hours the cotton cloth plates were examined but there was insufficient bacterial growth on the agar plates.

Method 3b - Modified method - Testing of Chitosan treatment of Cotton cloth

After the poor growth in method 3, the nutrient agar plates were inoculated using a 20µl pipette and the inoculum was spread around the agar using an inoculation loop in an attempt to produce better bacterial lawn growth.

There were zones of inhibition around the chitosan treated samples, but the bacterial growth was poor, so the experiment was repeated with fresh bacteria and fresh samples.

Repeat of method 3b

	Observation
MRSA 9551 Cotton cloth control	little growth, but up to edge of material
MRSA 9551 Chitosan treated cotton cloth	not very good growth, but still observable zone of inhibition
S. epidermis Cotton cloth control	Reasonable growth up to the edge of the material
S. epidermis Chitosan treated cotton cloth	Good growth. Observable zone of inhibition on three sides of the treated sample

Table 4.15 – chitosan treated cotton cloth results

There were zones of inhibition around the chitosan treated samples, but the bacterial growth was poor, so it was decided to repeat the experiment with fresh bacteria.

Method 3c - Modified method - Testing of Chitosan treatment of Cotton cloth

Method 3c is a modified version of method 3 as it contains a slightly expanded range of test samples. Some of the treated cotton cloth was kept un-sterilised to see if the high-pressure, high temperature steam has any effect on the bacteriostatic effect of the chitosan.

The chitosan did not adhere to the polypropylene as well as it did on the cotton cloth (polypropylene mesh is multifilament and knitted into an open structure and is not very wetable. The cotton cloth is a natural fibre woven structure and it is hydrophilic).

Sample	Observation
MRSA cotton cloth control (sterilised)	Good lawn growth up to and under cotton cloth sample
MRSA Chitosan treated cotton cloth (sterilised)	Good lawn growth. Zone of inhibition between 0.5mm and 3mm
MRSA Chitosan treated cotton cloth (not sterilised)	Good lawn growth. Zone of inhibition between 0.5mm and 7mm.
MRSA Chitosan treated polypropylene (sterilised)	Good lawn growth. Possible zone of inhibition less than 0.5mm.
Sample	Observation
S. *epidermis* cotton cloth control (sterilised)	Strong lawn growth up to and under cotton cloth sample
S. *epidermis* Chitosan treated cotton cloth (sterilised)	Good lawn growth. Zone of inhibition between 0.5mm and 3mm.
S. *epidermis* Chitosan treated cotton cloth (not sterilised)	Good lawn growth. Zone of inhibition between 0.2mm and 1.5mm.
S. *epidermis* Chitosan treated polypropylene (sterilised)	Good lawn growth. No zone of inhibition.

Table 4.16 – Chitosan coated cotton cloth and polypropylene results

The poor performance of the chitosan coated polypropylene could be due to the chitosan film failing to adhere to the hydrophobic polypropylene.

Method 3c (Modified method - Testing of chitosan treatment of cotton cloth) yielded some interesting data relating to the coating of cotton cloth with chitosan solution, with zones of inhibition observed. The polypropylene coated with chitosan had little to no observable effect on the bacteria. This study was derived partially to examine the potential for chitosan coatings for medical applications. One application could be as an anti infective coating for medical devices, but this could find a use as a spray-able coating for textiles within a hospital environment by reducing the ability of opportunistic pathogens to thrive on the clothes worn by hospital personnel and the soft furnishings found within a hospital environment, reducing potential transmission vectors. These applications are worthy of further study to determine their efficacy for the hospital environment.

Method 4 - Chitosan suspended in nutrient broth Vs MRSA

This method continues on from method 2c, but instead of using spectrophotometry to measure the bacterial growth, plate count agars were used along with serial dilutions of the broths at set time points to produce clearer data on the effect of chitosan in suspension in different quantities.

In this study, the chitosan samples are referred to as 0.01g, 0.008g, 0.006g, 0.004g, 0.002g and 0g. These refer to the w/v of chitosan in NB (where 0g is the control).

A plate count was done to find out how concentrated the inoculum was using a haemocytometer.

Cell (area on haemocytometer)	count (number of cells)
1	5
2	7
3	6
4	7
5	9
6	9
7	13
8	8
9	8
10	4
Mean	7.6

Table 4.17 - Cell count data for MRSA inoculum.

To convert this to cells per ml;

$7.6 \times (4 \times 10^{-6}) = 3.04 \times 10^{7}$ cells per ml in the initial inoculum

After 48 hours, the nutrient broths were observed. Upon observation, it was clear that the broths displayed some degree of variation in their visual appearance. As a

result, broths that appeared to be either more turbid or less turbid than the other broths of a certain chitosan concentration were not used for plate count purposes.

Sample	Sample No.	Observation
0.01g Chitosan	1	more turbid than the other two
0.008g Chitosan	2	less turbid than the other two
0.006g Chitosan	-	no variance
0.004g Chitosan	3	little less turbid than the other two
0.002 g Chitosan	2, 3	2 was more turbid, 3 was less turbid, 1 was in the middle
0g Chitosan (control)	-	no variance

Table 4.18 - Samples discounted on the basis of a difference in the broth appearance.

Sample	Sample No.
0.01g Chitosan	3
0.008g Chitosan	3
0.006g Chitosan	2
0.004g Chitosan	2
0.002 g Chitosan	1
0g Chitosan (control)	1

Table 4.19 - Samples used for the initial dilution

	Dilution		
Sample	10-6	10-7	10-8
Control (0g)	tmtc	110	9
0.002g	0	0	4
0.004g	tmtc	181	20
0.006g	461	64	6
0.008g	180	86	-
0.01g	tmtc	111	0

Table 4.20 - Results from 48 hours. Tmtc – too many to count

The results from 48 hrs shows an unusual result for 0.002g and it is likely to be caused by human error.

	Dilution		
Sample	10-6	10-7	10-8
Control (0g)	ng	1049	ng
0.002g	3	ng	ng
0.004g	ng	ng	ng
0.006g	ng	ng	ng
0.008g	ng	ng	ng
0.01g	ng	ng	ng

Table 4.21 - Results from 72 hours. Ng – no growth

This result was unexpected. The control, only one PCA grew and showed an abundance of bacteria. The 0.002g, only one plate grew and showed a marked decrease in culture density. There are four possible explanations for this. 1. Problem with the culture media, 2. Human error or 3. (discounting the control) that the viable bacterial population had decreased to such a level, that the dilutions did not contain enough bacteria or 4. Phenomena as yet not understood.

Further to this unexpected result, the broth cultures used the day before were kept and stored in the 20ºC incubator in case of such problems. When they were examined, they had changed appearance. Some of the broths had almost lost their turbidity, whilst others had a clear section at the top of the broth as if the media had separated. Realising the relevance of this, the results were noted so they could be correlated to the findings.

Sample	Repeat	Observation
Control (0g)	1	Medium turbidity. Can see through
Control (0g)	2	Medium turbidity. Can see through
Control (0g)	3	Medium turbidity. Can see through
0.002g	1	The lower 6/7th of the broth = very turbid, can't see through. Top 1/7th, very clear. Upon disturbance, turbid layer settles back, leaving the clear top 1/7th
0.002g	2	The lower 6/7th of the broth = very turbid, can't see through. Top 1/7th, very clear. Upon disturbance, turbid layer settles back, leaving the clear top 1/7th
0.002g	3	Medium turbidity. Can see through
0.004g	1	Medium turbidity. Can see through
0.004g	2	Medium turbidity. Can see through
0.004g	3	The lower 6/7th of the broth = very turbid, can't see through. Top 1/7th, very clear. Upon disturbance, turbid layer settles back, leaving the clear top 1/7th
0.006g	1	Medium turbidity. Can see through
0.006g	2	A little more turbid. Can see through
0.006g	3	Medium turbidity. Can see through
0.008g	1	0.008 1 and 0.008 3 look identical. Both are very turbid. Top 1/7th is not clear, but appears to be starting to clear.
0.008g	2	Medium turbidity. Can see through
0.008g	3	0.008 1 and 0.008 3 look identical. Both are very turbid. Top 1/7th is not clear, but appears to be starting to clear.
0.01g	1	very Turbid – opaque
0.01g	2	Medium turbidity. Can see through
0.01g	3	Quite turbid. Can still see through

Table 4.22 - Observations of broth appearance

In discussion with a colleague it was explained that MRSA changes from Gram +ve to Gram –ve when a colony reaches a certain age. Further studies will be performed with fresh cultures of MRSA.

Method 4b - Modified method - Chitosan suspended in nutrient broth Vs MRSA

Method 4 was modified to include a more appropriate selection of dilutions for inoculating the plate count agar.

The graphs on the next few pages show growth rates over time of MRSA challenged by chitosan in varying quantities.

The raw data has been excluded from the results section. The full results can be found in appendix A.3, method 4b.

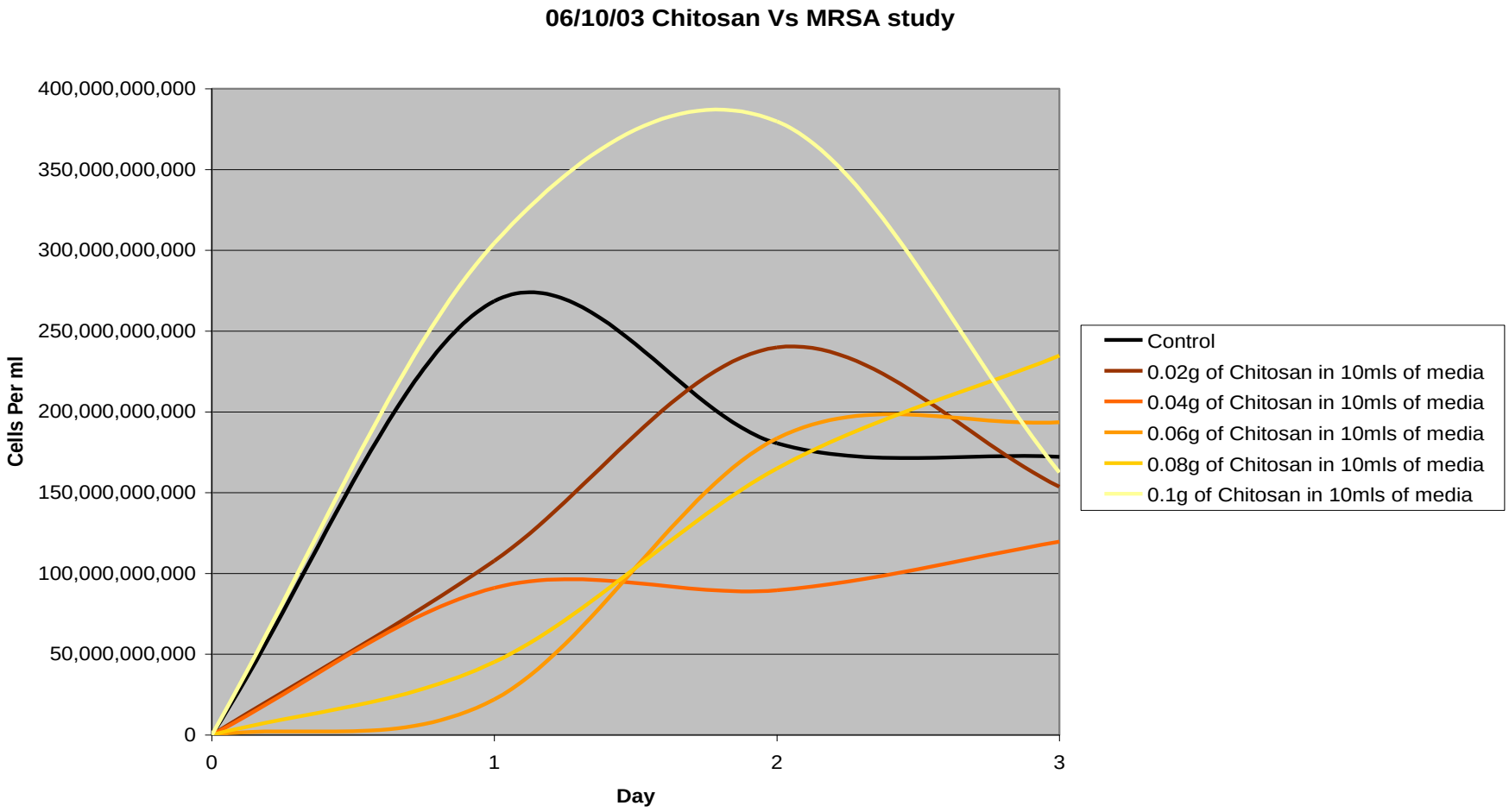


Fig. 4.23 - Graph of average growth of MRSA Vs Chitosan.

Looking at fig. 4.23 there is one sample that shows improved growth over the control, and the other 4 indicating lower growth than the control. The 0.1g sample is clearly showing improved growth and the reasons for this are unknown. The initial inoculum for this experiment was approximately 3.85×10^7 cells (1.93×10^8 cells per ml), and the maximum viable number of cells in the control during the experiment was approximately 2.69×10^{11} cells per ml.

Looking at the samples between 0.02g and 0.08g, we see two interesting features. Firstly, none of these samples contain bacterial growth greater than the control. Secondly, the growth rate is considerably slowed. The slow growth could be due to the bacteriostatic effect of chitosan inhibiting the slowing the growth of the bacteria. With a slowed growth of the bacteria, one may assume that the bacteria would still reach the abundance found in the control, but what we see is that the bacteria decline at lower abundance than the control. This might suggest that chitosan increases the auto toxic effect of the bacteria, preventing them reaching the numbers of the control and causing them to die at lower bacterial concentrations.

With the 0.1g sample out growing the control, it could be that there was an error in the experiment or that chitosan is most effective at a certain concentration and may even be metabolised by the bacteria when out with that concentration.

No graph could be produced for the chitosan and MRSA data from 20/10/03. This was due to the sample dilutions not falling within the countable range.

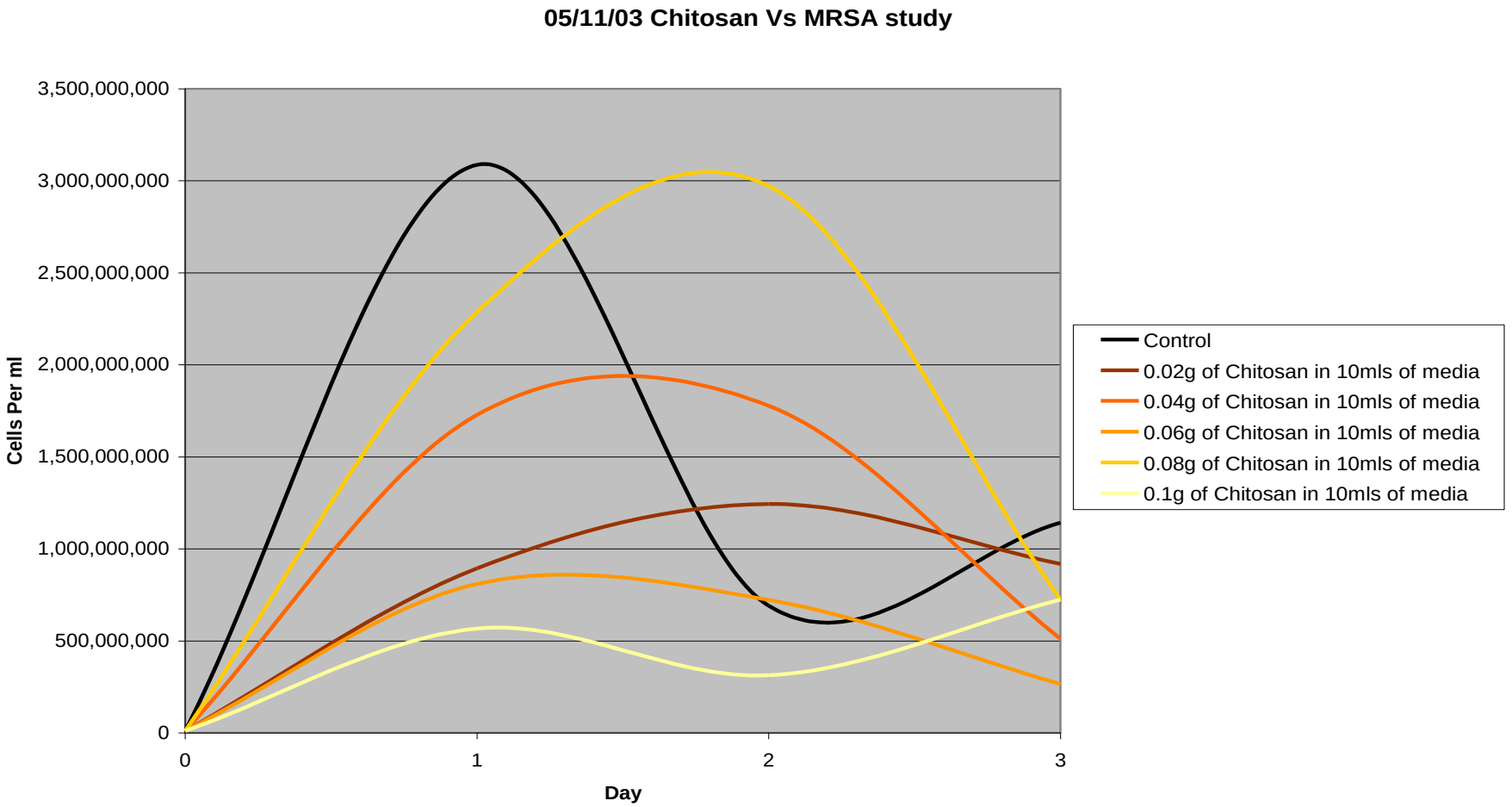


Fig. 4.24 - Graph of average growth of MRSA Vs Chitosan.

Looking at fig 4.24 we can see that all of the chitosan samples show lower growth than the control. The initial inoculum for the 05/11/03 experiment was approximately 9.76x 10^6 cells (4.88x 10^7 cells per ml), and the maximum viable number of cells in the control during the experiment was approximately 3.09 x 10^9 cells per ml.

Looking at this graph, we see that none of the samples containing chitosan develop the same number of bacteria as the control, although the 0.08g sample comes near and the 0.1g sample shows the lowest growth. The only obvious difference between this experiment is the concentration of the inoculum, which is ~1/4 the concentration used in the 06/10/08 study. It would therefore appear that the efficacy of chitosan is related in some manner to the quantity of bacteria used for the initial inoculation.

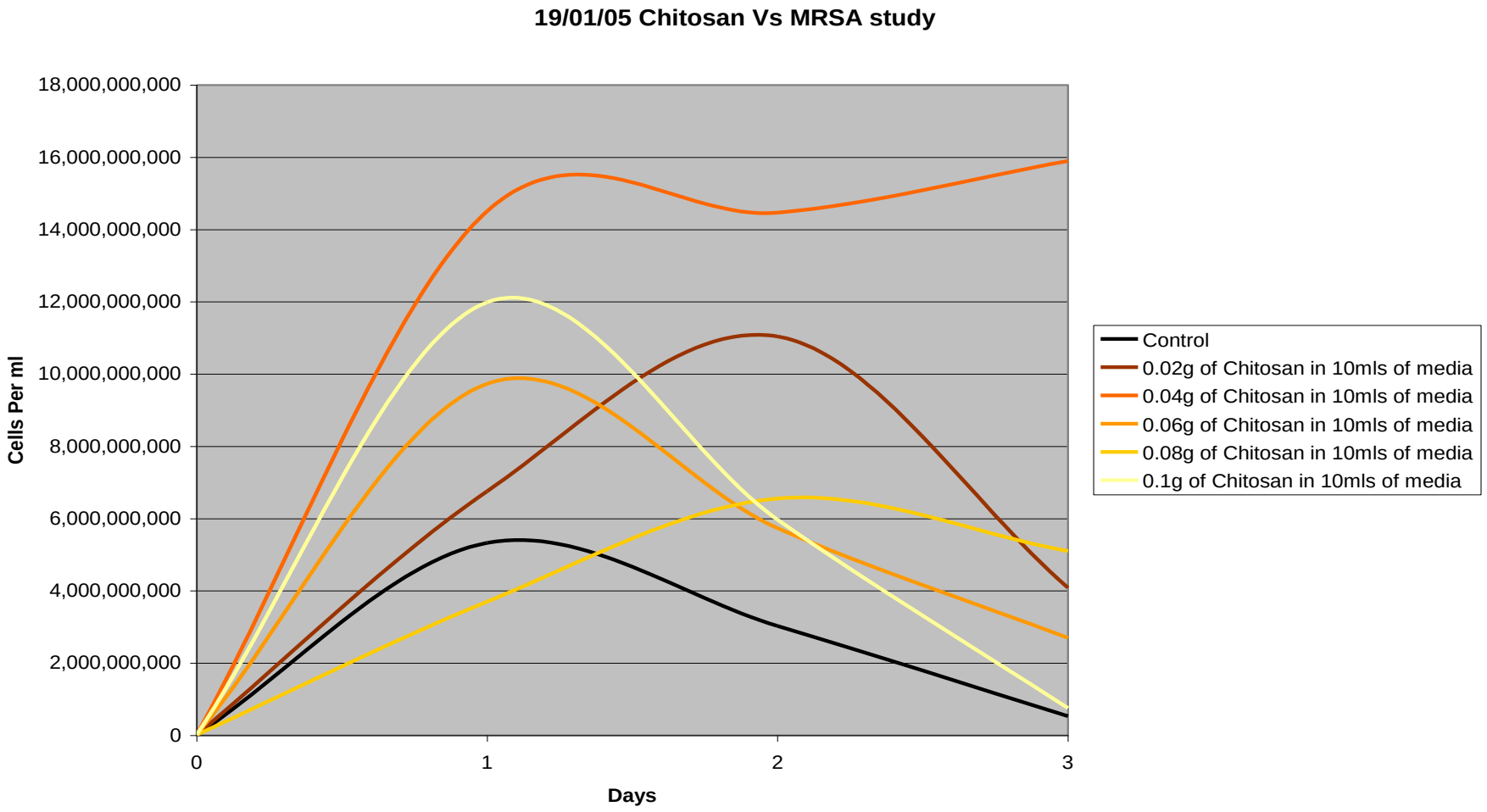


Fig. 4.25 - Graph of average growth of MRSA Vs Chitosan.

Fig 4.25 does not show the same trend as the previous two graphs (fig 4.23 and 4.24). The initial inoculum for this experiment was approximately 1.88 x 10^7 cells (9.40 x 10^7 cells per ml), and the maximum viable number of cells in the control during the experiment was approximately 5.33 x 10^9 cells per ml.

This data does not appear to correlate with the two previous graphs. It could be human error but it is more likely that there is some phenomenon occurring that is as yet unknown. The only known variable is that this experiment contained half the inoculum of the 06/10/03 experiment and double the inoculum of the 05/11/03 experiment. The question as to whether this is a factor in the variation seen in the results can only be addressed by further study.

4.2 Production of Biomaterial Samples

The polyurethane and polyester were fabricated externally (commercially available materials). These samples were cut into sample sizes and plasma treated. The polypropylene, poly-ε-caprolactone and Solanyl were extruded at Heriot-Watt University as described in the methodology section. The PLA was to be extruded but no useable tape could be produced, therefore it was cast as a film instead.

The plasma treatment was to be performed on the Europlasma equipment using argon, oxygen and ammonia, but due to a technical fault with the equipment, alternative equipment was used (the Nanotech equipment). Only argon and ammonia gas were available for the Nanotech plasma equipment. As some samples had been treated on the Europlasma equipment, they were included in the tissue culture study.

4.3 Sample Characterisation

4.3.1 Differential scanning calorimetry (DSC) Analysis

The DSC images display the energy required to increase the temperature of the sample over a range of temperatures versus time. The dips and spikes in the energy

profile correlate to the polymer sample proceeding through different phases. The lowest dip is the melting point of the polymer.

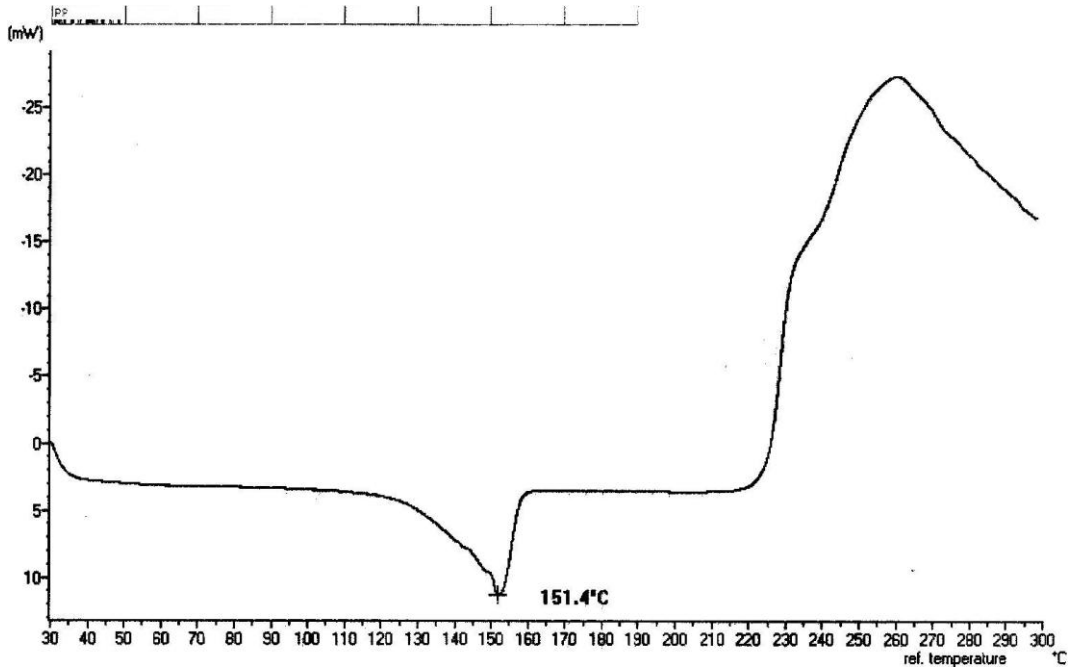


Fig. 4.26 - Polypropylene DSC. Melting point 151.4ºC

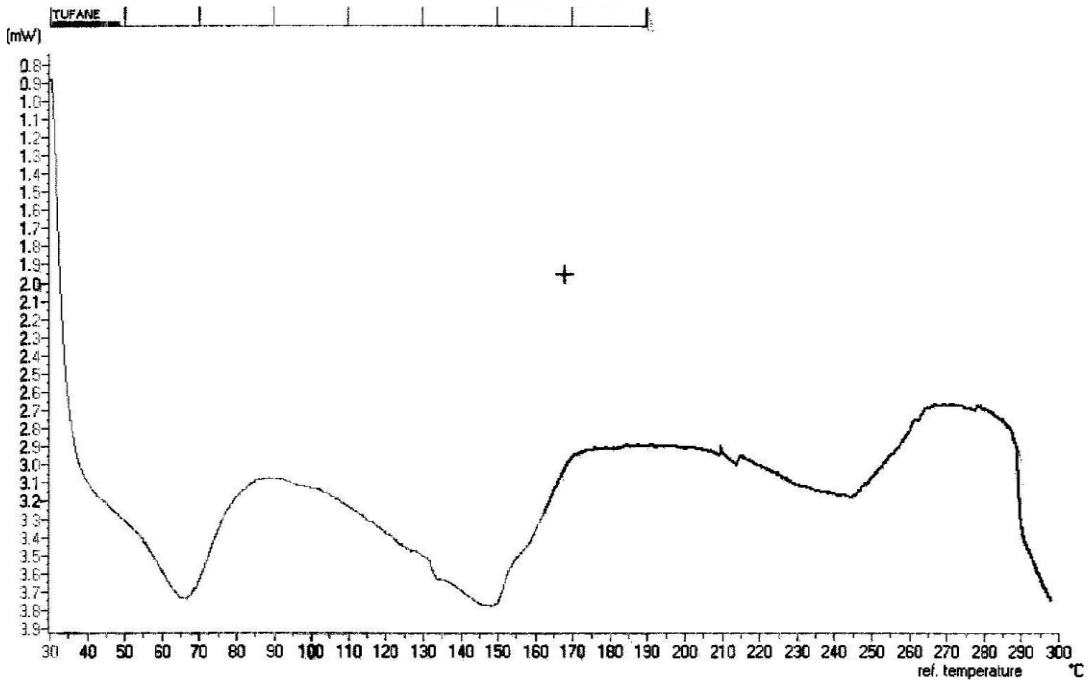


Fig. 4.27 - Polyurethane DSC. Melting point 149.5ºC

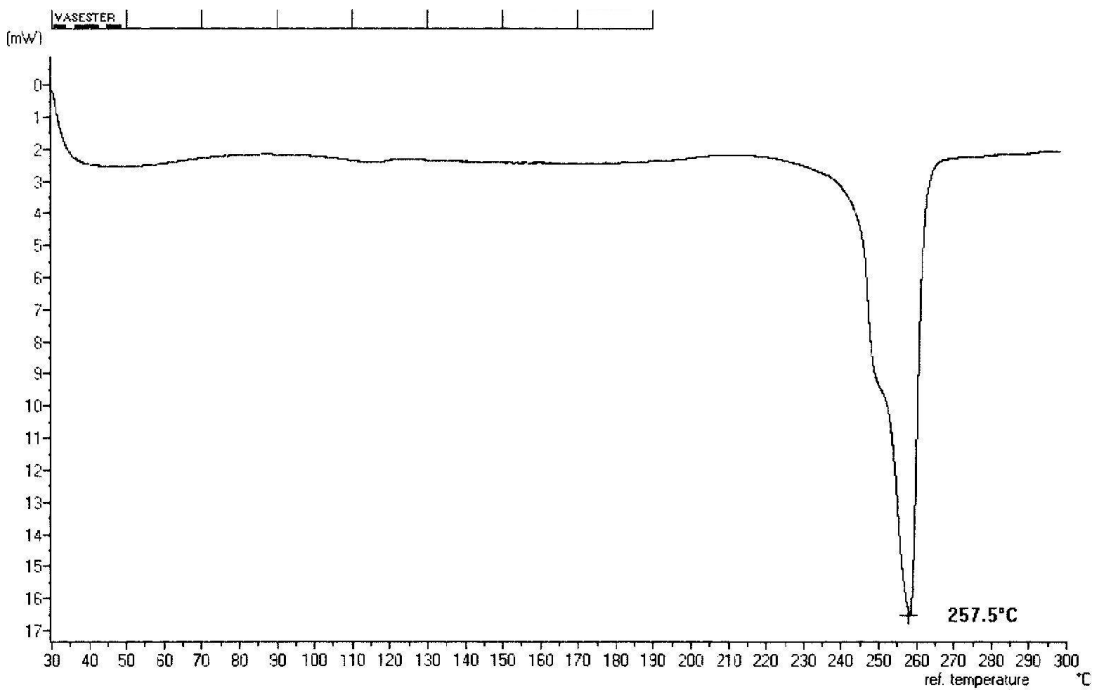


Fig. 4.28 - Polyester DSC. Melting point 257.5ºC

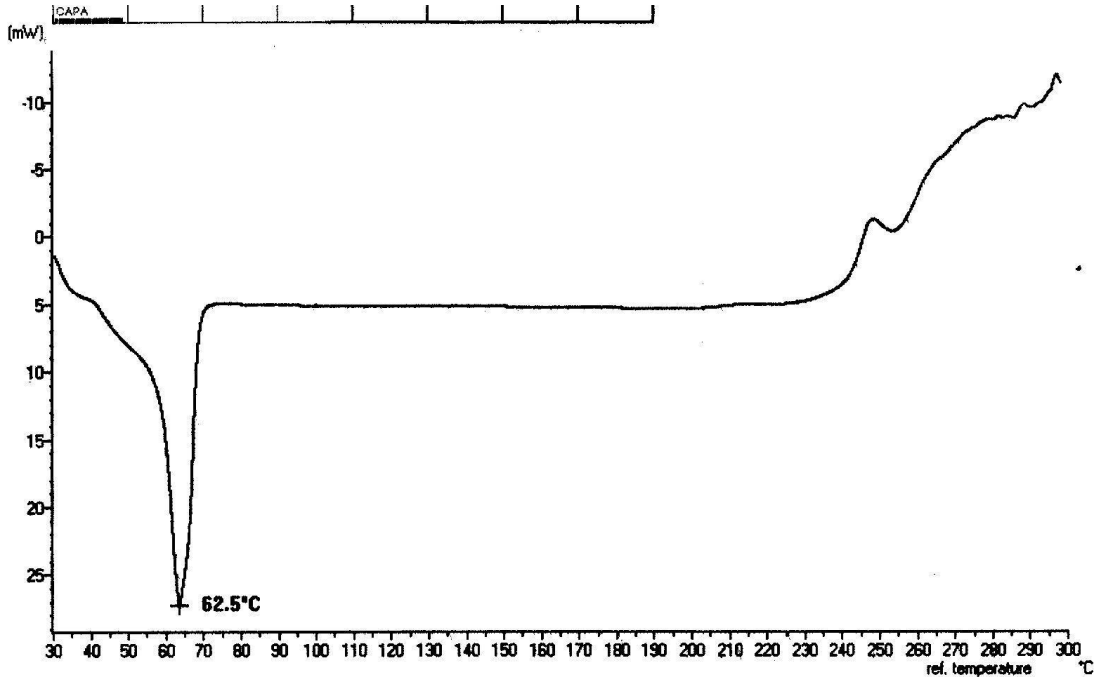


Fig. 4.29 - Polycaprolactone DSC. Melting point 62.5ºC

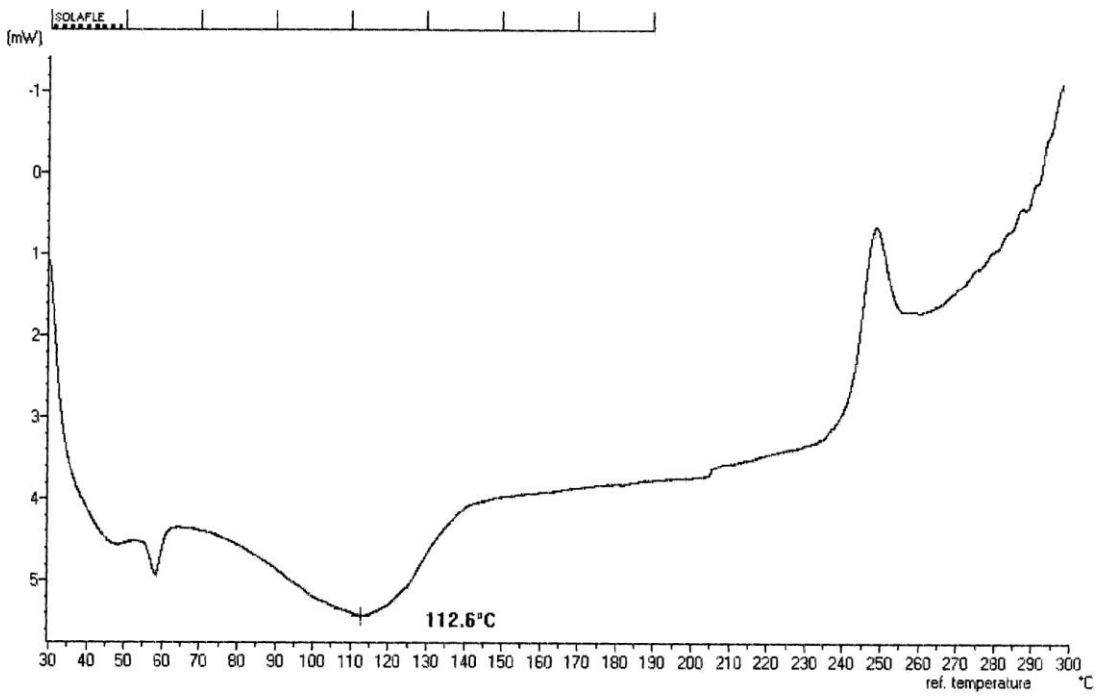


Fig. 4.30 - Solanyl Flexibilitis DSC. Melting point 112.5ºC

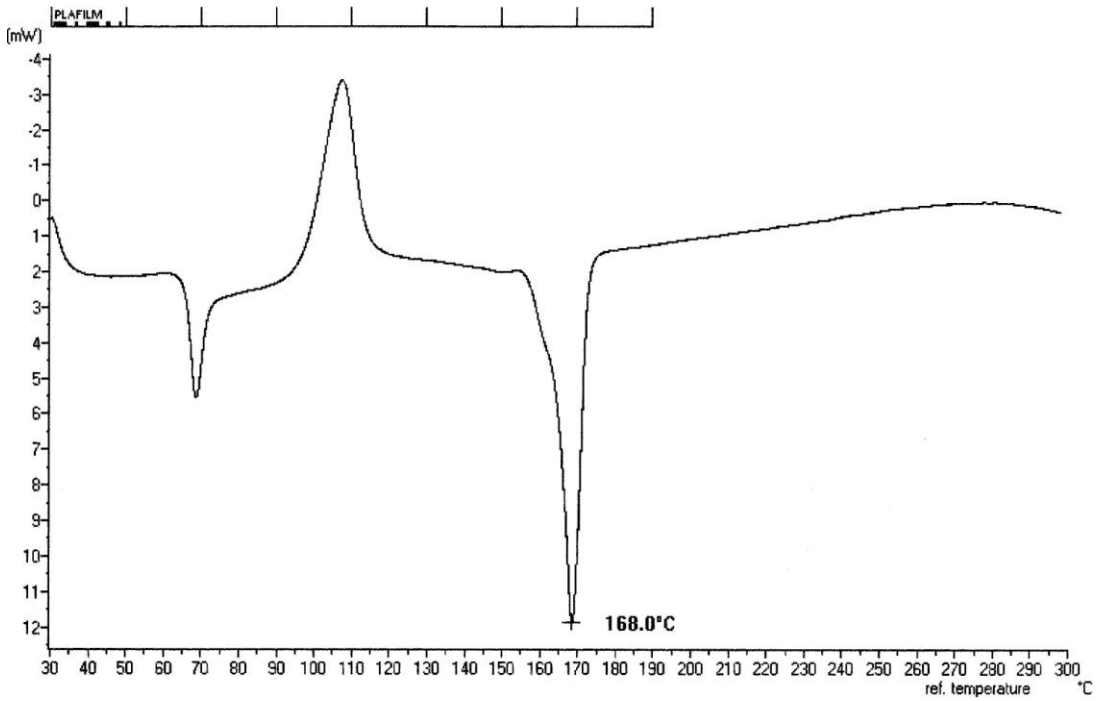


Fig. 4.31 - Poly-L-Lactic Acid DSC. Melting point 168.0ºC

4.3.2 SEM Analysis

The samples were analysed by scanning electron microscope to examine the surface for change after plasma treatment and to illustrate differences in surface morphology. Some of the materials have a very plain surface but they are included to illustrate the difference in the surfaces of the biomaterials.

Artefacts are visible in some of the SEM images (dust ect).

Polypropylene

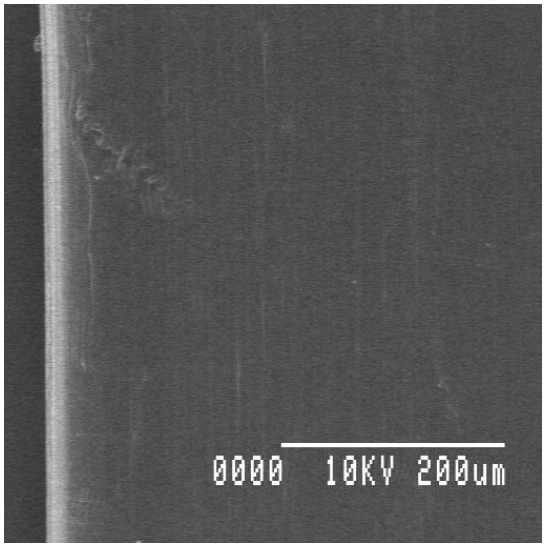


Fig. 4.32 - Polypropylene control. Very plain surface with few surface grooves produced during extrusion

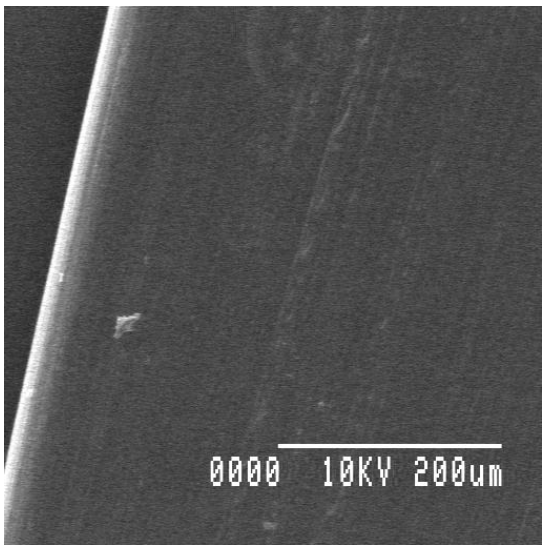


Fig. 4.33 - Argon Plasma Treated Polypropylene (Nanotech). At this magnification, there is no visual difference between this polypropylene and the control polypropylene.

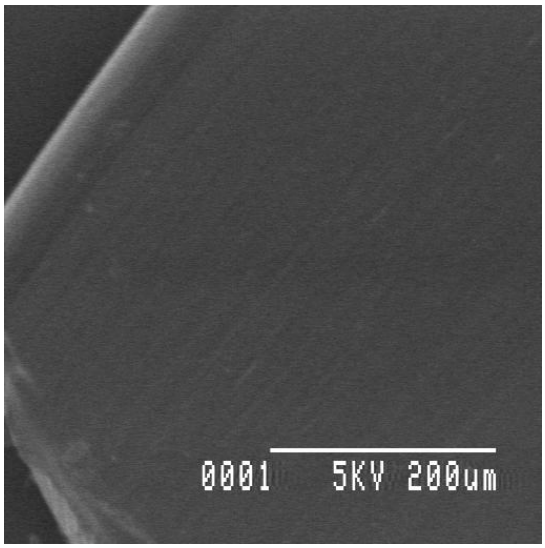


Fig. 4.34 - Ammonia Plasma Treated Polypropylene (Nanotech). At this magnification, there is no visual difference between this polypropylene and the control polypropylene.

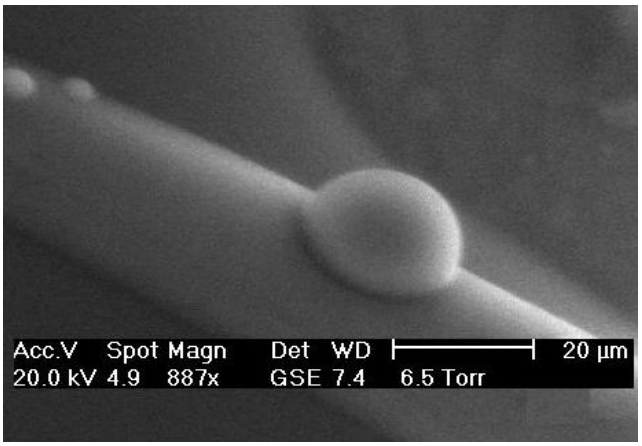


Fig 4.35 - Spherical cap shapes of water on untreated fibre surface.

Source (Wei et al., 2004)

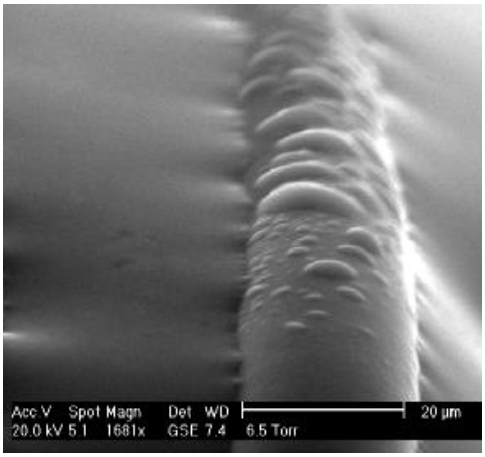


Fig 4. 36 - Growth and coalescence of water droplets on plasma treated PP fibre surface.

Source (Wei et al., 2004)

Fig 4.35 is an environmental SEM image of untreated polypropylene and Fig 4.36 is an environmental SEM image of oxygen plasma treated polypropylene. These images illustrate that although there is no visual difference in the surface, the hydrophilicity of the sample in fig. 4.36 is greater than the sample in fig. 4.35.

Tuftane Polyurethane

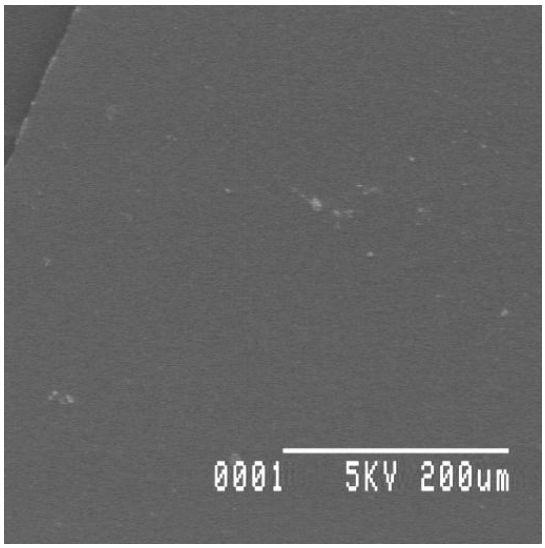


Fig. 4.37 - Tuftane Polyurethane. This material has a very smooth surface.

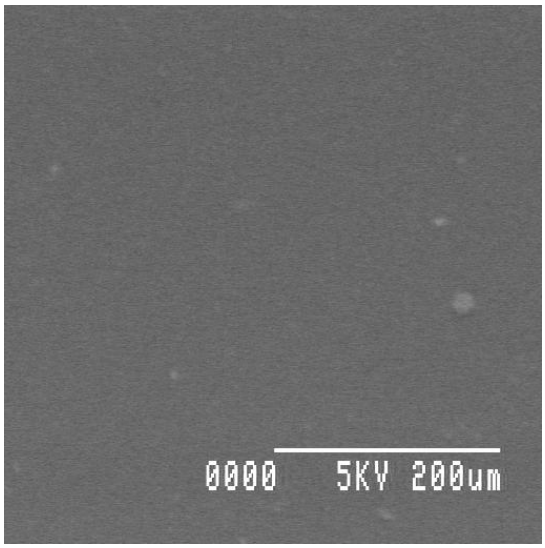


Fig. 4.38 - Argon Plasma Treated Tuftane Polyurethane (Nanotech). At this magnification, there is no visual difference between this polyurethane and the control polyurethane.

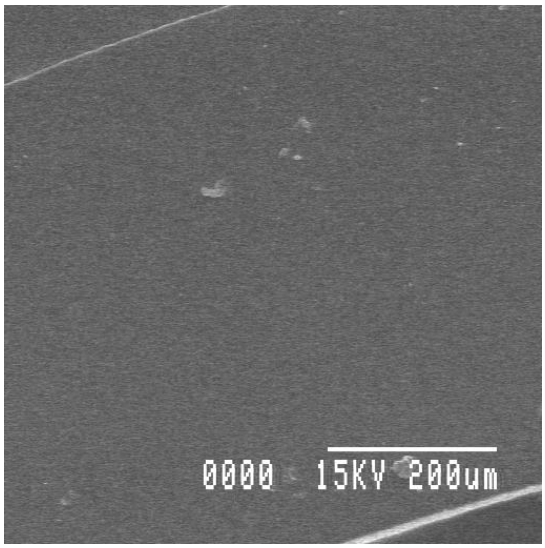


Fig. 4.39 - Ammonia Plasma Treated Tuftane (Nanotech). At this magnification, there is no visual difference between this polyurethane and the control polyurethane.

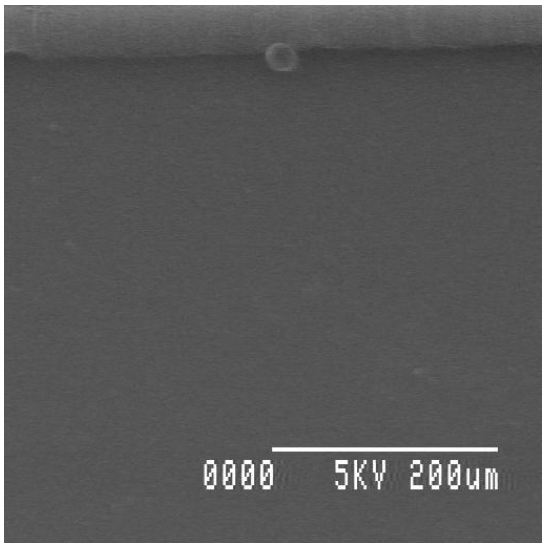


Fig. 4.40 - Argon Plasma Treated Tuftane Polyurethane (Europlasma). At this magnification, there is no visual difference between this polyurethane and the control polyurethane.

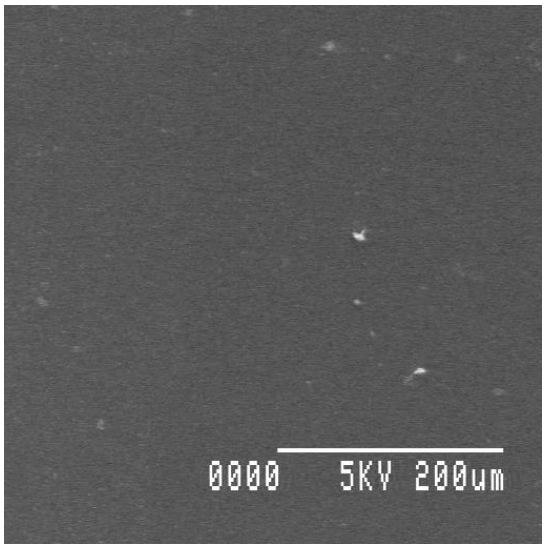


Fig. 4.41 - Oxygen Plasma Treated Tuftane Polyurethane (Europlasma). At this magnification, there is no visual difference between this polyurethane and the control polyurethane.

Vascutek polyester

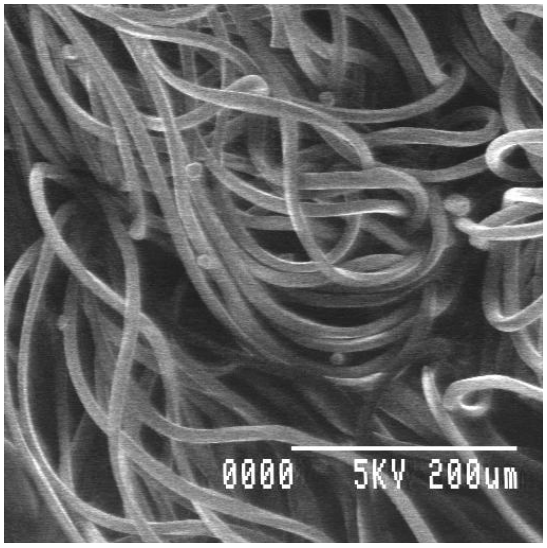


Fig. 4.42 - Vascutek Polyester control. The structure of this material is very different to the other materials therefore no direct comparisons may be made between this material and the others.

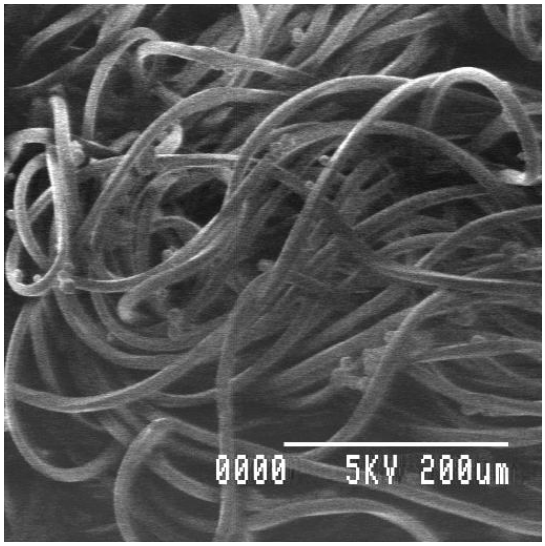


Fig. 4.43 - Argon Plasma Treated Vascutek polyester (Nanotech). At this magnification, there is no visual difference between this polyester and the control polyester.

Fig. 4.44 - Ammonia Plasma Treated Vascutek polyester (Nanotech). At this magnification, there is no visual difference between this polyester and the control polyester.

Poly-ε-Caprolactone 6400

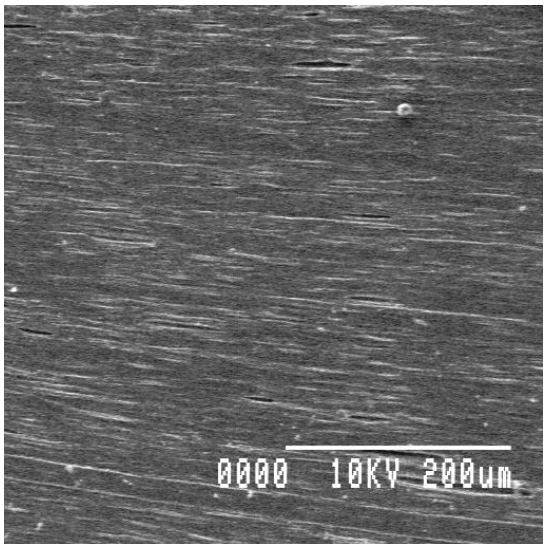


Fig. 4.45 - Poly-ε-Caprolactone 6400 control. This material displays a grooved surface.

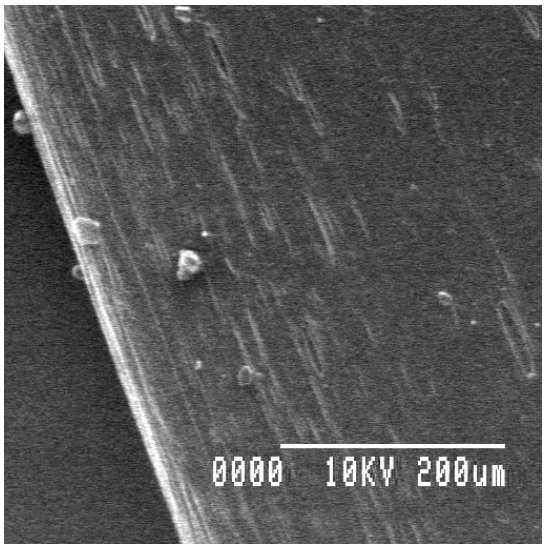


Fig. 4.46 - Argon Plasma Treated Poly-ε-Caprolactone 6400 (Nanotech). At this magnification, there is no visual difference between this Poly-ε-Caprolactone and the control Poly-ε-Caprolactone.

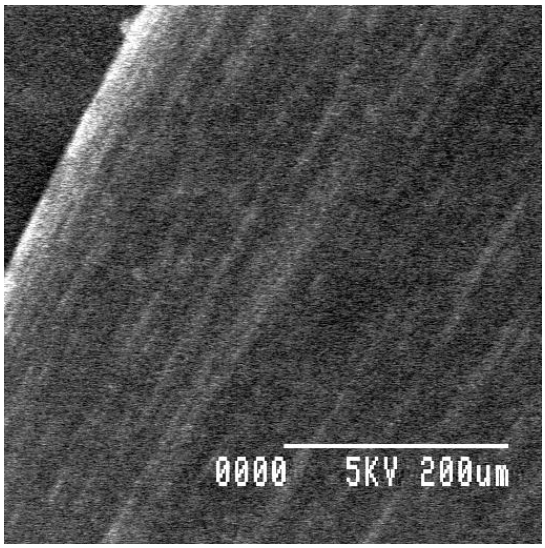


Fig. 4.47 - Ammonia Plasma Treated Poly-ε-Caprolactone 6400 (Nanotech). At this magnification, there is no visual difference between this Poly-ε-Caprolactone and the control Poly-ε-Caprolactone.

Solanyl

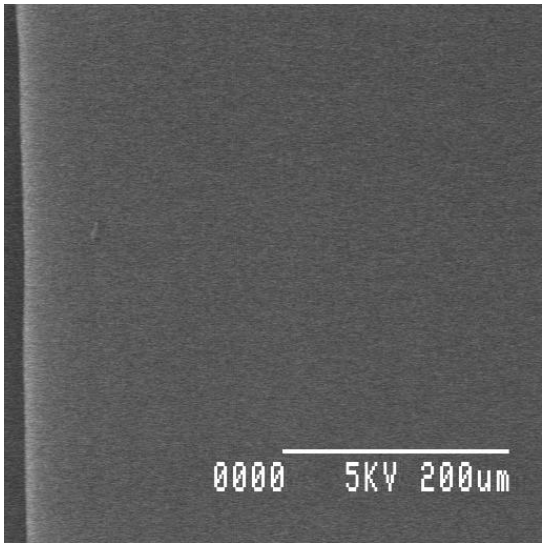


Fig. 4.48 - Solanyl Control. This material has a very smooth surface.

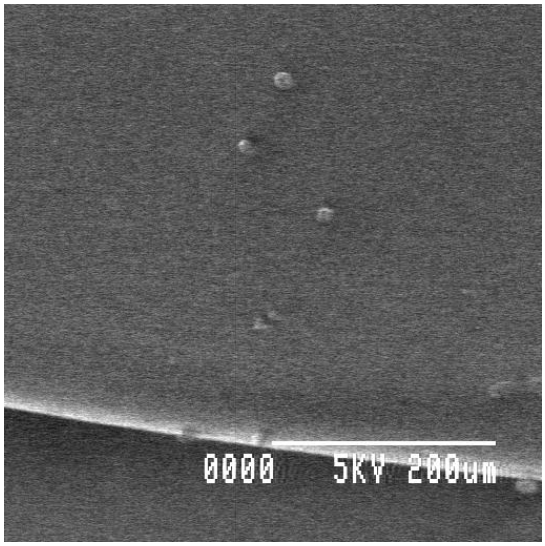


Fig. 4.49 - Argon Plasma Treated Solanyl (Nanotech). At this magnification, there is no visual difference between this Solanyl and the control Solanyl.

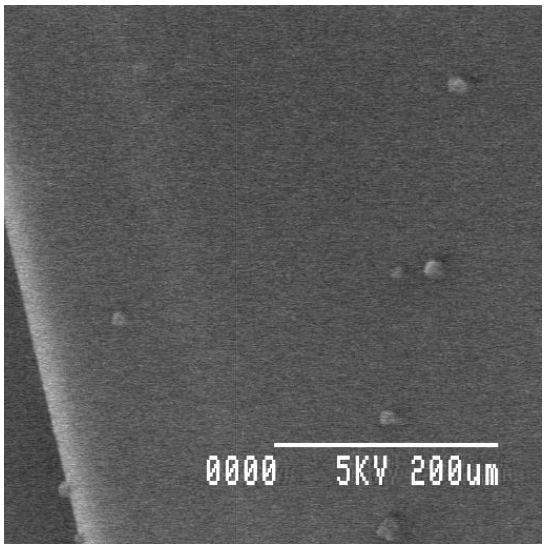


Fig. 4.50 - Ammonia Plasma Treated Solanyl (Nanotech). At this magnification, there is no visual difference between this Solanyl and the control Solanyl.

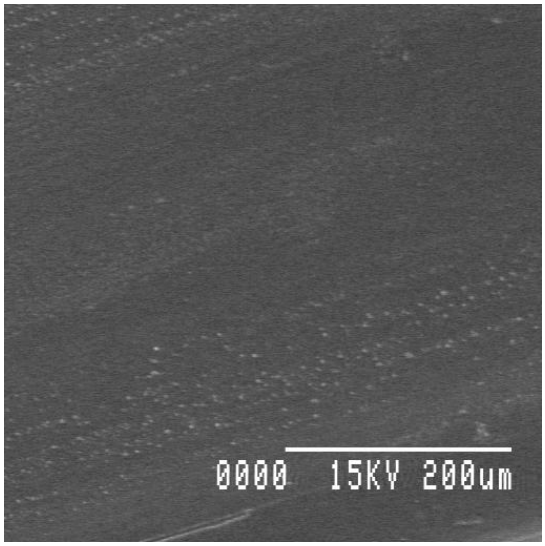


Fig. 4.51 - Solanyl extruded with 2% chitosan (w/w). The fine bumps in this image were interpreted as chitosan powder

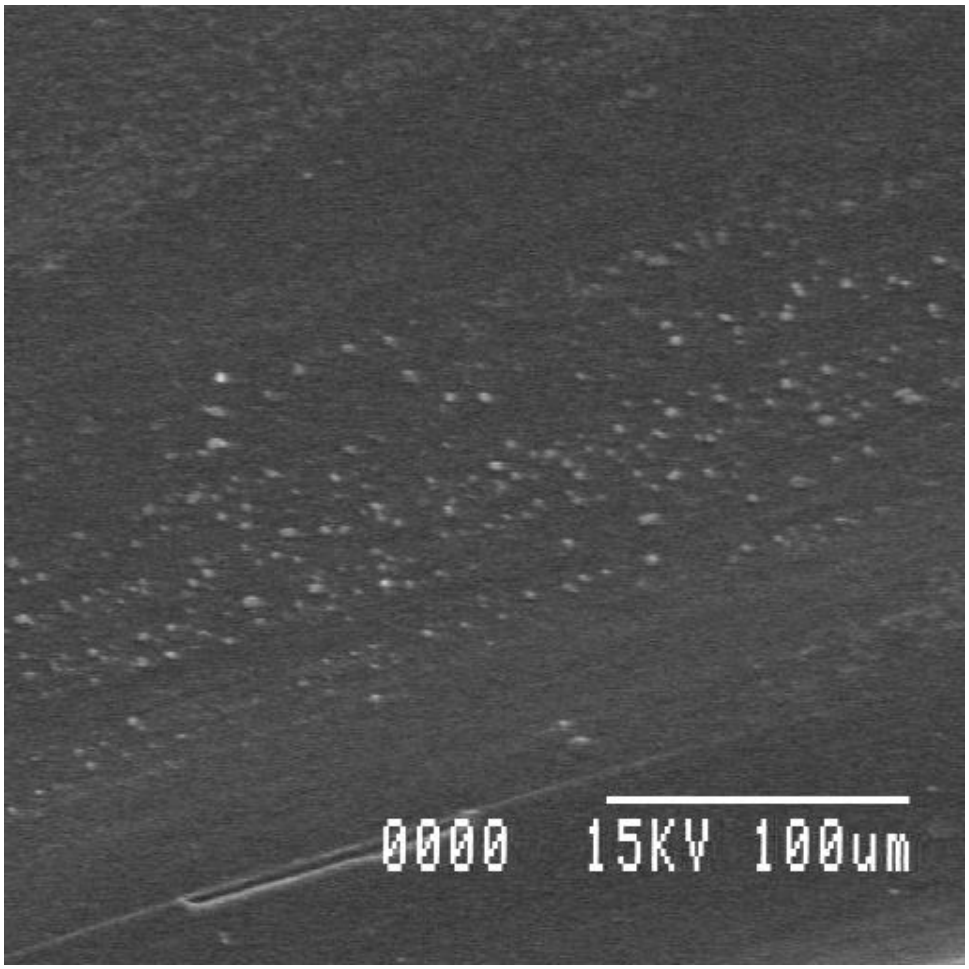


Fig. 4.52 - Solanyl extruded with 2% chitosan (w/w). The fine bumps can be seen more clearly in this image.

Fig. 4.52 shows the chitosan powder incorporated into the Solanyl tape.

Poly-l-lactic acid

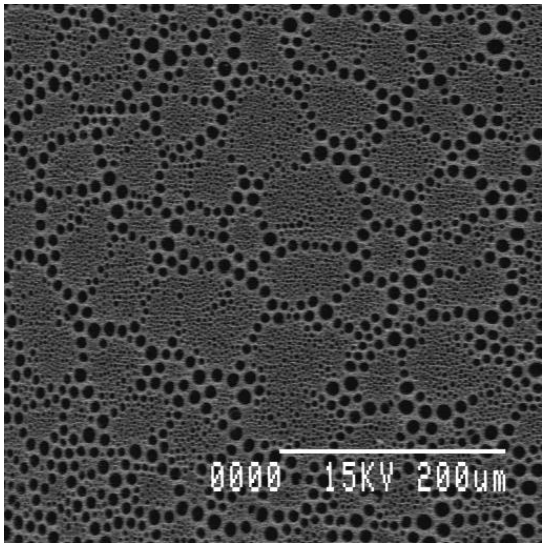


Fig. 4.53 - Poly-l-lactic acid Control. The highly porous structure can be seen in this image. It is assumed that this structure is due to the solvent casting method of production (Chun et al., 2000).

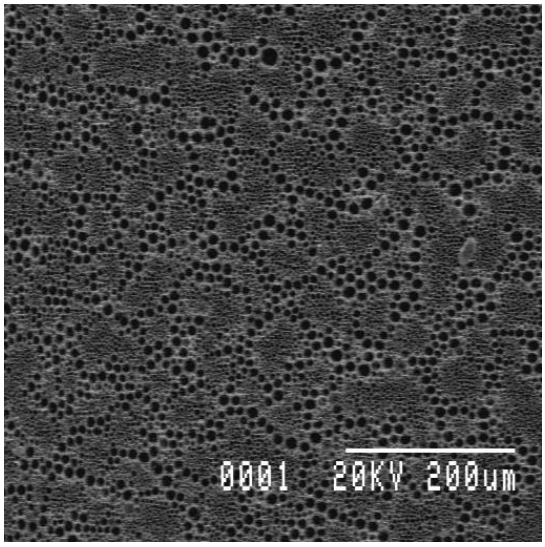


Fig. 4.54 - Argon Plasma Treated Poly-l-lactic acid (Nanotech). At this magnification, there is no visual difference between this Poly-l-lactic acid and the control Poly-l-lactic acid.

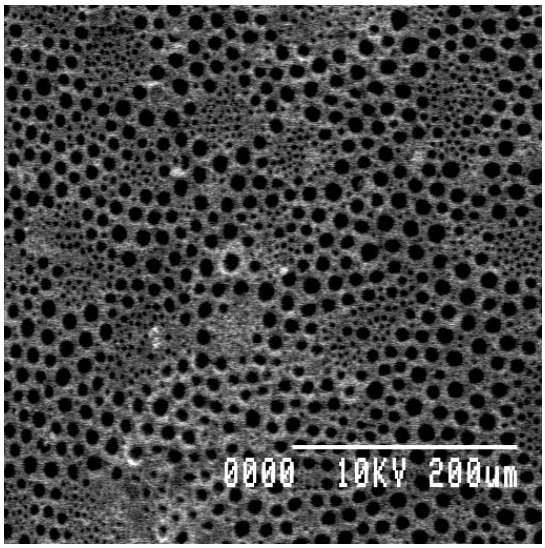


Fig. 4.55 - Ammonia Plasma Treated Poly-l-lactic acid (Nanotech). At this magnification, there is no visual difference between this Poly-l-lactic acid and the control Poly-l-lactic acid.

Analysis of Pore Size of PLA Sample

With the PLA displaying a highly porous structure, measurement of the pore size was performed. The following images were used in the measurements as they were deemed representative of the PLA pore size. The pore sizes were calculated assuming the pores were circular. The area of the pores was calculated using the equation (4.1).

Area of a circle = π x Diameter Equation (4.1)

The measurements were converted to scale using the scale bars in the SEM images. For pores where only half was visible in the image, the area was halved.

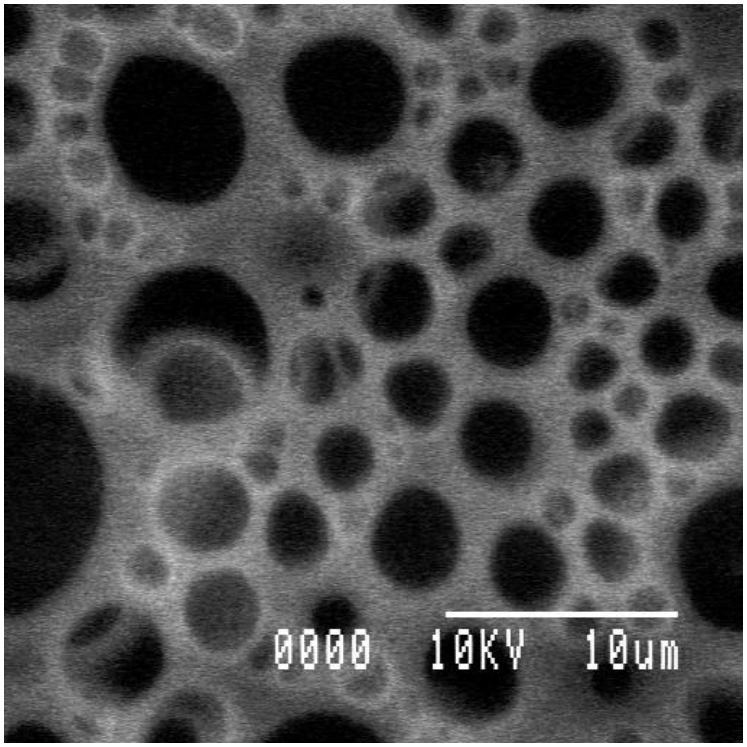


Fig. 4.56 This image is the first of the PLA images to be measured and therefore will be referred to as PLA 1.

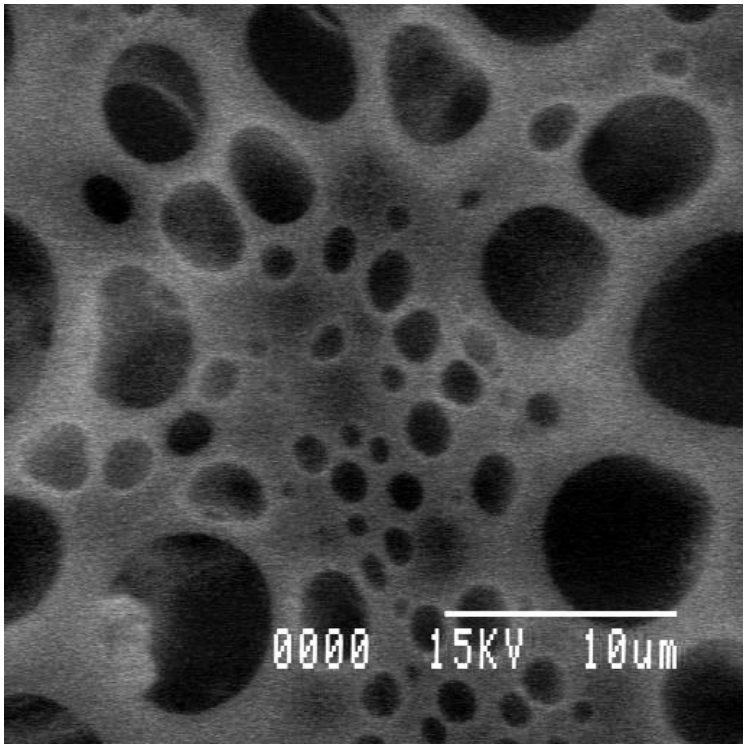


Fig. 4.57 This image is the second of the PLA images to be measured and therefore will be referred to as PLA 2.

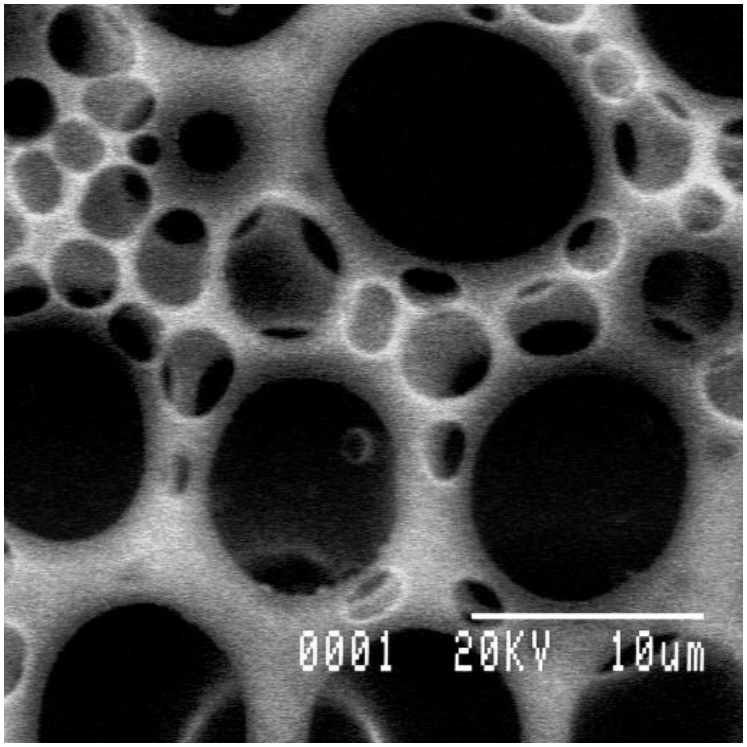


Fig. 4.58 This image is the third of the PLA images to be measured and therefore will be referred to as PLA 3.

Pore Size Measurements of Figures 4.55 – 4.57

The PLA images were printed out and the dimensions of the pores were measured using a ruler. The scale bar in the images was used to convert the measurements from cm to μm. The complete measurement data can be found in appendix B.

	PLA 1	PLA 2	PLA 3
Average pore area (μm^2)	7.04	7.44	12.22
Total area of pores in image (μm^2)	563.03	402.03	464.37
Percentage porosity	55.80%	39.84%	46.02%
Average area of pores for the three images (μm^2)			8.90
Average percentage of pores for the three images			47.22%

Table 4.59 Summary of PLA pore size measurements.

The result of these measurements was that the PLA had pores between 0.13μm^2 to 39.58 μm^2, with the average pore size at 3.31 μm^2 and a percentage area of pores of 17.4%.

4.4 Tissue Culture Study

The following images help illustrate the difficulty with measuring the growth accurately. The use of cellular stains was avoided to prevent potential detrimental effects on cell growth.

The next 2 pages show photographs of the tissue culture samples through a microscope. These photographs are for illustration only.

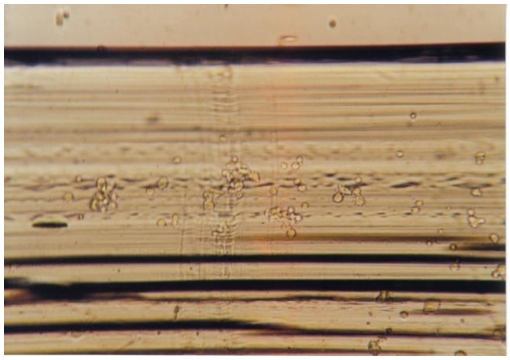


Fig. 4.60

Polypropylene

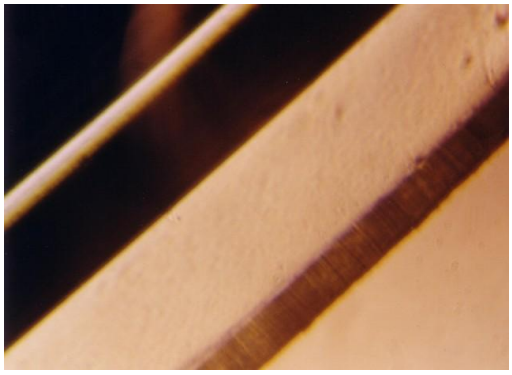


Fig. 4.61

Polyurethane

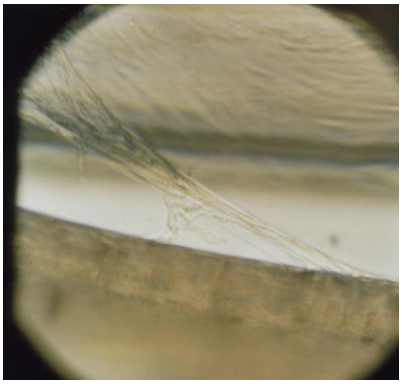


Fig. 4.62

Polyurethane

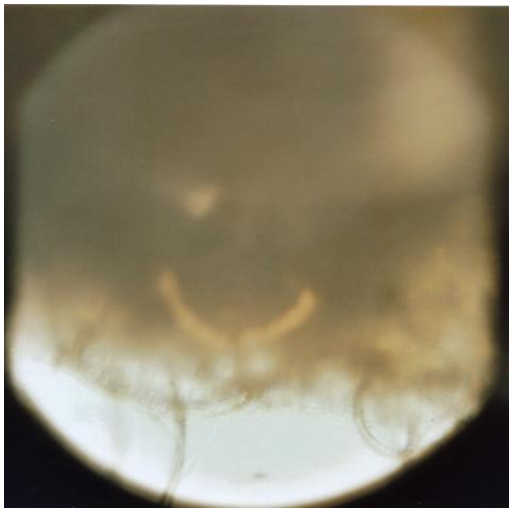


Fig. 4.63

Polyester

Fig. 4.64

Polyester

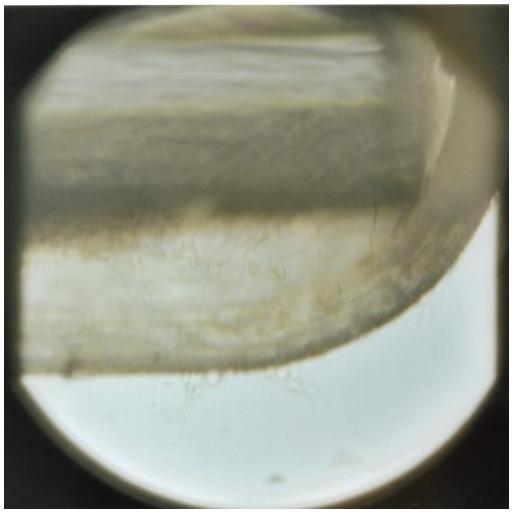


Fig. 4.65

Poly-ε-caprolactone

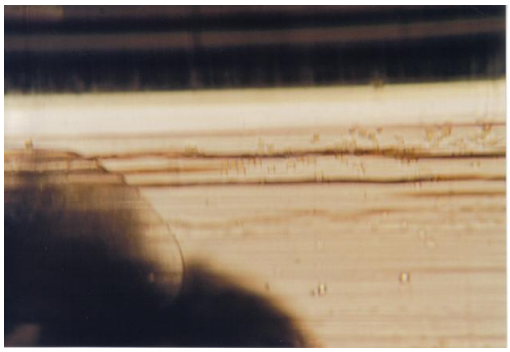


Fig. 4.66

Solanyl

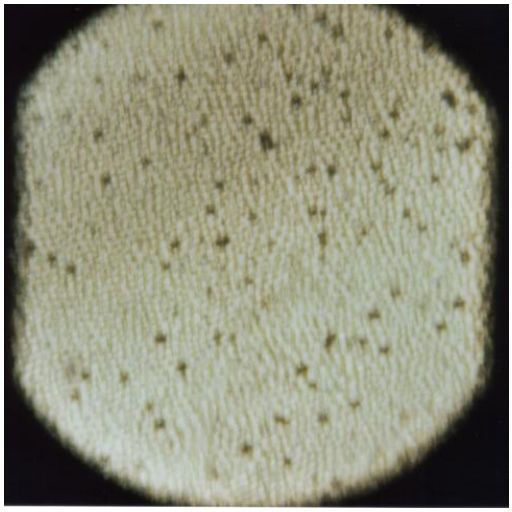


Fig. 4.67

Poly-L-Lactic acid

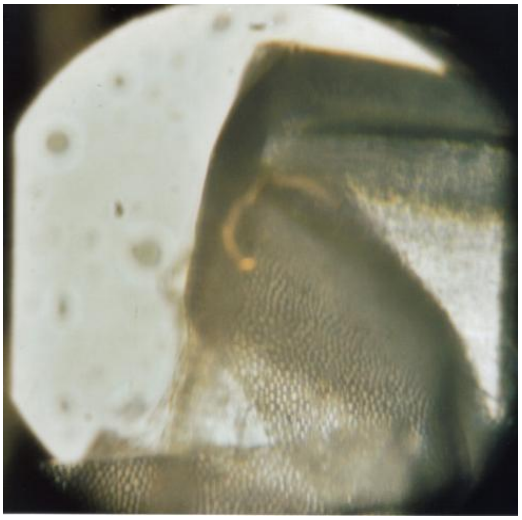


Fig. 4.68

Poly-L-Lactic acid

Figures 4.60 through to 4.68 illustrate what was seen down the microscope when measuring the MRC-5 cell growth along the samples. These images are a mixture of demonstrating the material as seen through the microscope combined with attempts to photograph the cells growing on the material clearly. These images were taken using a 35mm SLR with a microscope mount.

When the samples were examined weekly, measurements of growth along the samples were recorded. In addition, cells could sometimes be seen growing on the glass sample support or the tissue culture flask. This was recorded and the data can be seen in appendix C.2. The colour of the media was recorded to provide supporting evidence for the growth measurements. This can also be seen in appendix C.2.

All of the materials tested comprised a control, an argon treated material and an ammonia treated material. For some materials, other treatments were included. Both Solanyl and polypropylene were treated with a chitosan solution both with and without plasma treatment. In addition, some one off treatments were tested.

These treatments were; argon treated polyurethane (on the Europlasma machine), oxygen plasma treated polyurethane (on the Europlasma machine), chitosan powder sprinkled on to a sample of polyurethane prior to argon plasma treatment (Nanotech) and Solanyl extruded with 2% chitosan powder (w/w).

The oxygen and argon samples were created using the Europlasma equipment. The equipment failed shortly after these treatments and therefore alternative equipment was used for the other samples. The argon treatment provided a comparison between the two different plasma treatment machines, while the oxygen plasma treatment can only be directly related to the Europlasma treated argon sample and the control. The chitosan powder sprinkled on to a sample of polyurethane prior to argon plasma treatment sample was made to determine whether plasma could be used to attach powders to surfaces and to compare this method with the addition of chitosan film. It was then used in the study to determine if the chitosan powder would persist on the material and to see what effect it might have on cell growth.

The following pages show graphs that chart the growth of the cells along the samples over a period of 29 days. Each sample was replicated 9 times, although not every sample produced a clear result. See the appendix C.1 for further details.

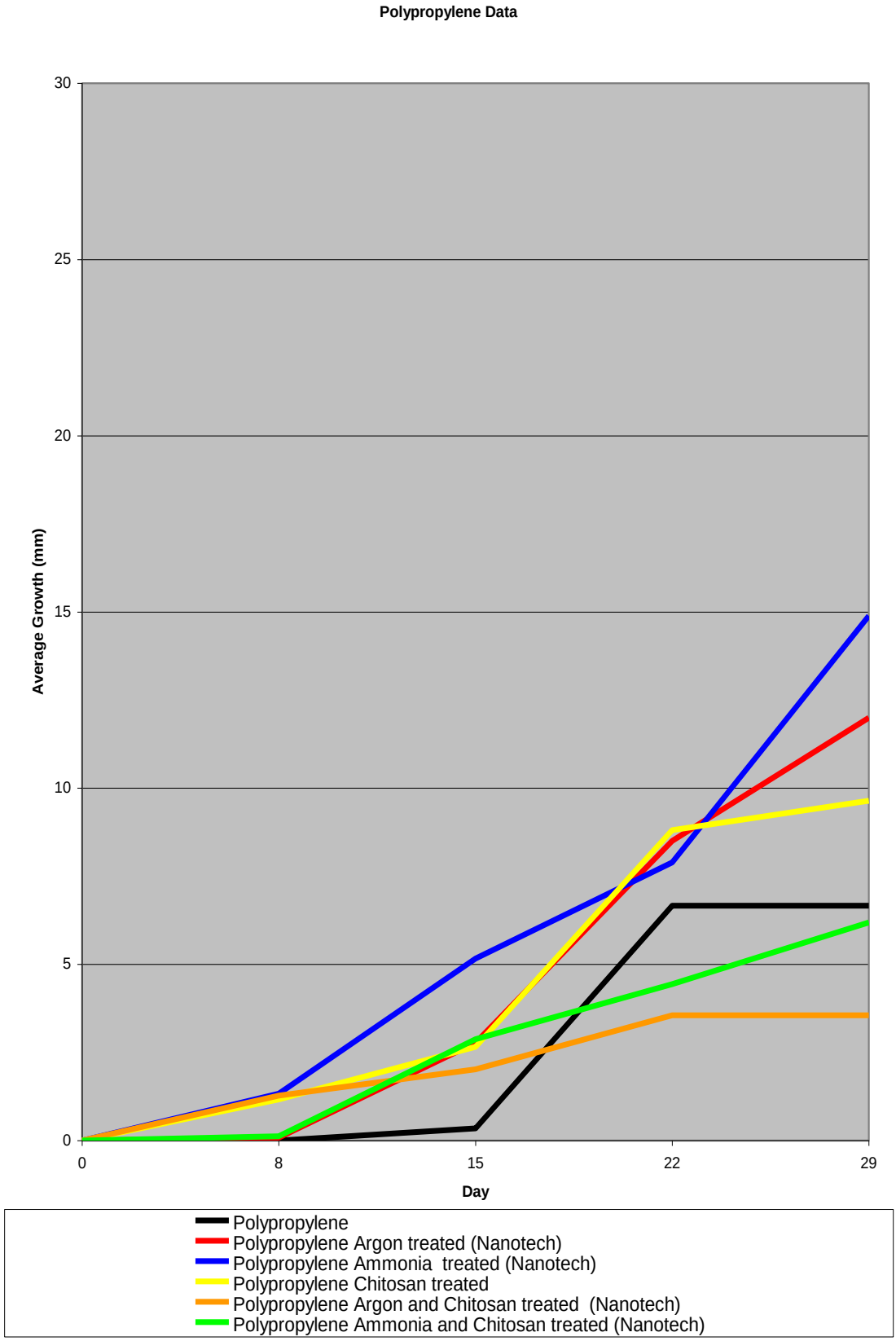


Fig. 4.69 - Graph of average growth of fibroblasts on the polypropylene samples.

Fig 4.69 shows the growth of MRC-5 cells along the polypropylene samples. Looking at the first 15 days of growth, it is clear that the untreated polypropylene performs the worst for supporting initial growth of the cells. The majority of the other treatments seem to perform better, supporting 2-2.5mm of growth on the samples (polypropylene argon treated, polypropylene chitosan treated, polypropylene argon chitosan treated and polypropylene ammonia chitosan treated). The best material over the initial 15 days was the polypropylene ammonia treated. This data indicates that any of the treated materials performs better than native polypropylene for supporting growth over 15 days.

Over the next 15 days, the data shows a change in the growth rate of he MRC-5 cells on the samples. The polypropylene samples with gas plasma treatment and chitosan coating maintain a steady growth rate but perform poorly when compared to the other samples. The unmodified polypropylene displays a sharp increase in growth up to day 22 and then displays no further growth over the remaining 7 days. The polypropylene samples with single treatments (chitosan, argon and ammonia) display sustained growth, out performing the native polypropylene, although the chitosan coating growth rate appears to be tailing off over the last 7 days. The slowing of the chitosan coated polypropylene sample could in part be due to the hydrophobic polypropylene on which the coating was applied. The chitosan coating may be partial, with sections of no coating. The plasma treated samples show sustained growth, due in part to a consistent surface modification which enhances the polypropylene hydrophilicity.

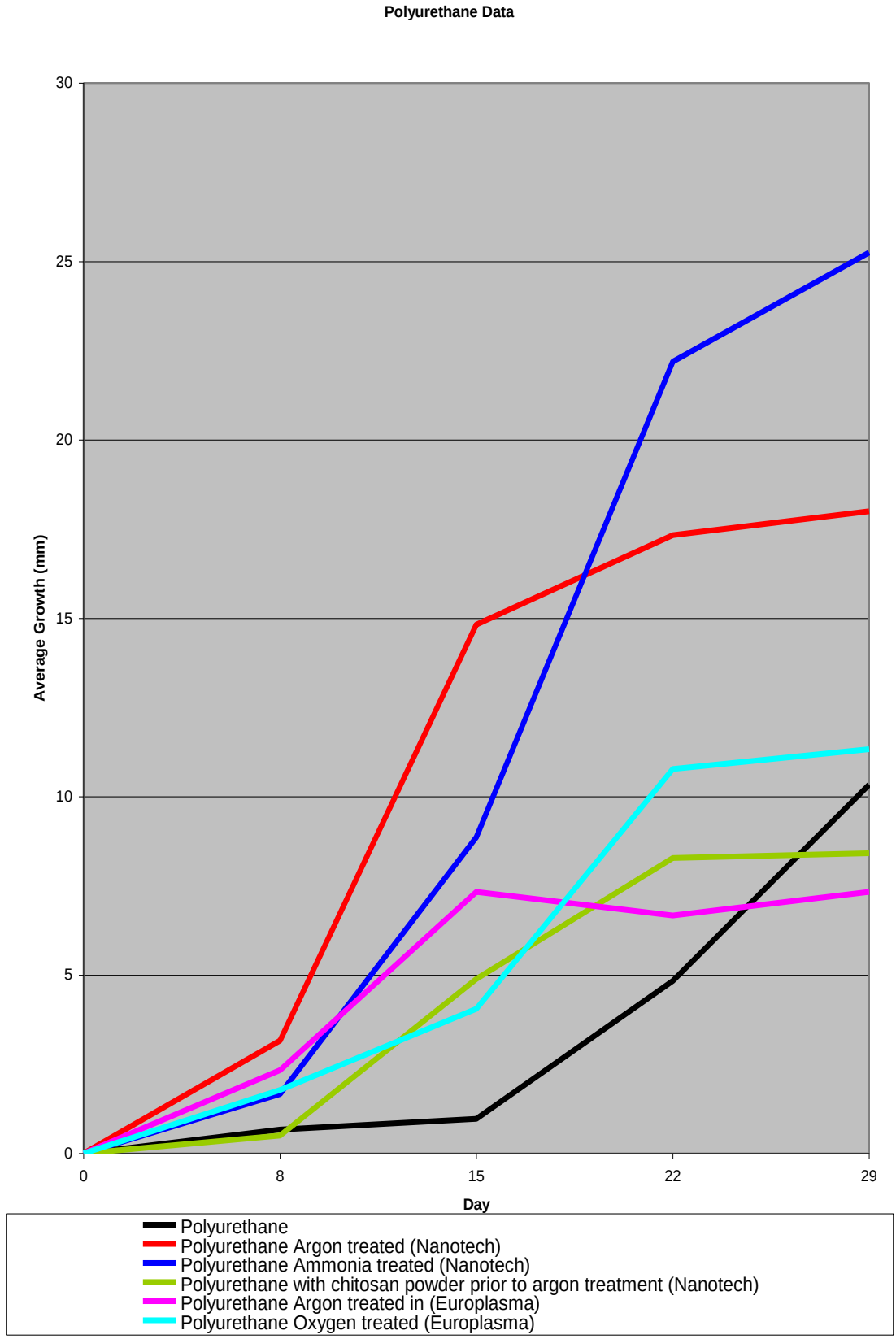


Fig. 4.70 - Graph of average growth of fibroblasts on the polyurethane samples.

Fig. 4.70 shows the growth of MRC-5 cells along the Polyurethane samples. This selection of treatments includes plasma treatment from two different plasma treatment machines; therefore they shall be differentiated by manufacturer of the control equipment (Nanotech and Europlasma). Looking at the first 15 days of growth, it is clear that the untreated polyurethane is out-performed by the modified polyurethane samples. The oxygen plasma treated polyurethane (Europlasma) and the polyurethane sprinkled with chitosan powder prior to argon treatment were both displaying greater growth rates than the untreated polyurethane over the first 15 days.

The two Nanotech treated samples and the Europlasma argon sample perform very well, although the difference in growth rate between the two argon treatments is interesting, with the Nanotech sample encouraging twice the growth of the Europlasma samples. This could be partially due to differences in the plasma chamber. The Nanotech chamber was far smaller than the Europlasma chamber and as a direct result, although the gas was in excess, and the other parameters were matched as closely as possible, the distance between the plates (between which the RF frequency was discharged) was far smaller, therefore producing a more focused plasma discharge. This hypothesis will need to be confirmed in a later study.

In the latter 14 days of the study it can be seen that the control sample and the oxygen plasma treated sample perform similarly, while the argon and chitosan sample and the Europlasma argon treated sample are outperformed by the control. This was an unexpected outcome.

In contrast, the two samples treated on the Nanotech equipment, the argon and ammonia samples dramatically outperform the control, with the ammonia again performing the best with an average growth ~15mm greater than the control. The two Nanotech samples also perform considerably better than the polypropylene samples with the corresponding treatments. The results also show that the polyurethane control outperforms the polypropylene control.

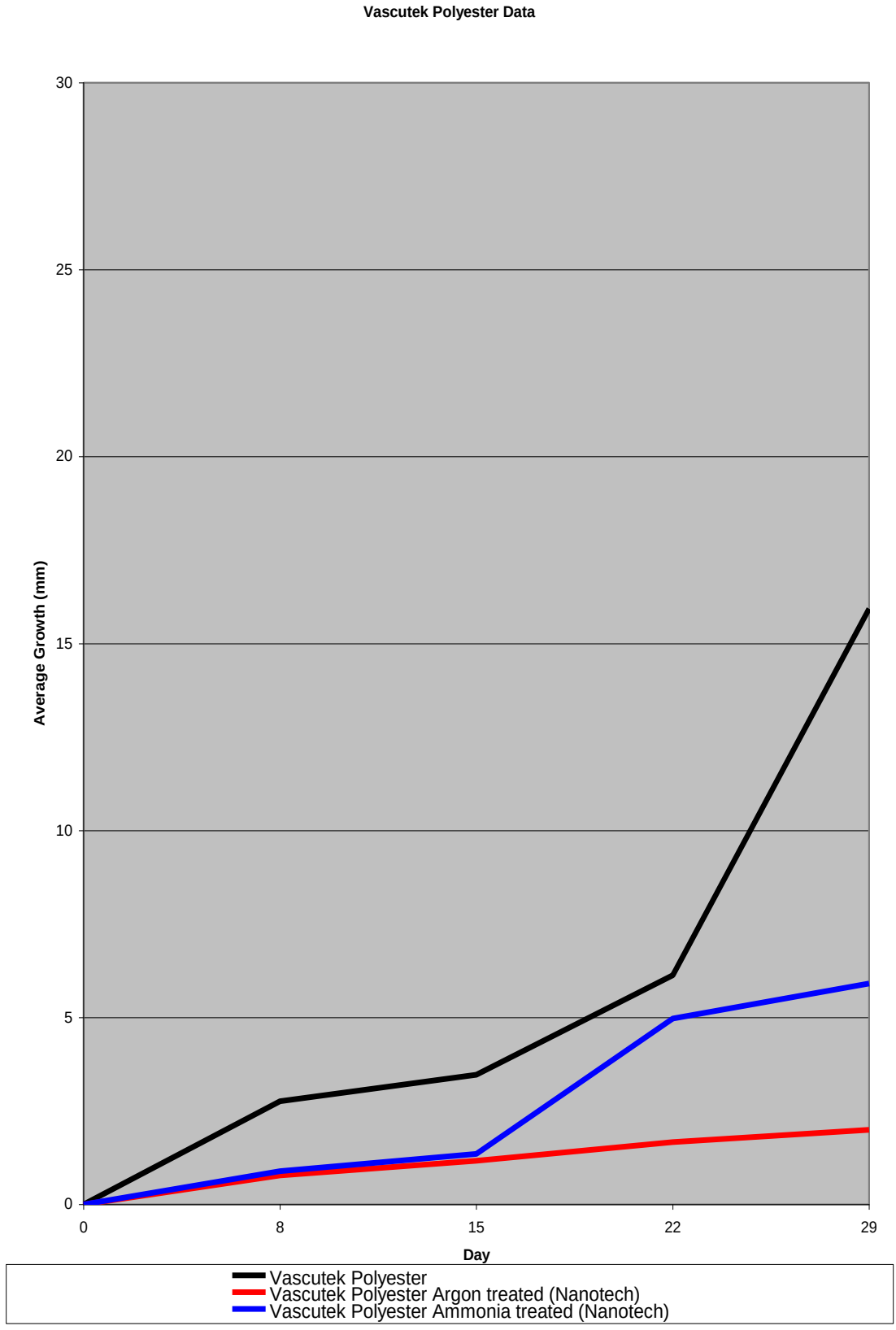


Fig. 4.71 - Graph of average growth of fibroblasts on the polyester samples.

Fig. 4.71 shows the growth of MRC-5 cells along the Vascutek samples. This graph shows the control material out performing both of the plasma treated samples. This material is used as a vascular prosthesis and when adding cells to the samples, the cells could be seen to wick into the sample very quickly, demonstrating the hydrophilicity. By plasma treating the samples, it is possible that the hydrophilicity was reduced resulting in lower growth on the samples. Although the plasma treated samples do not perform as well as the control, it can still be seen that the ammonia plasma treated sample performs better than the argon plasma treated sample, although for the first 15 days, the growth rate is similar.

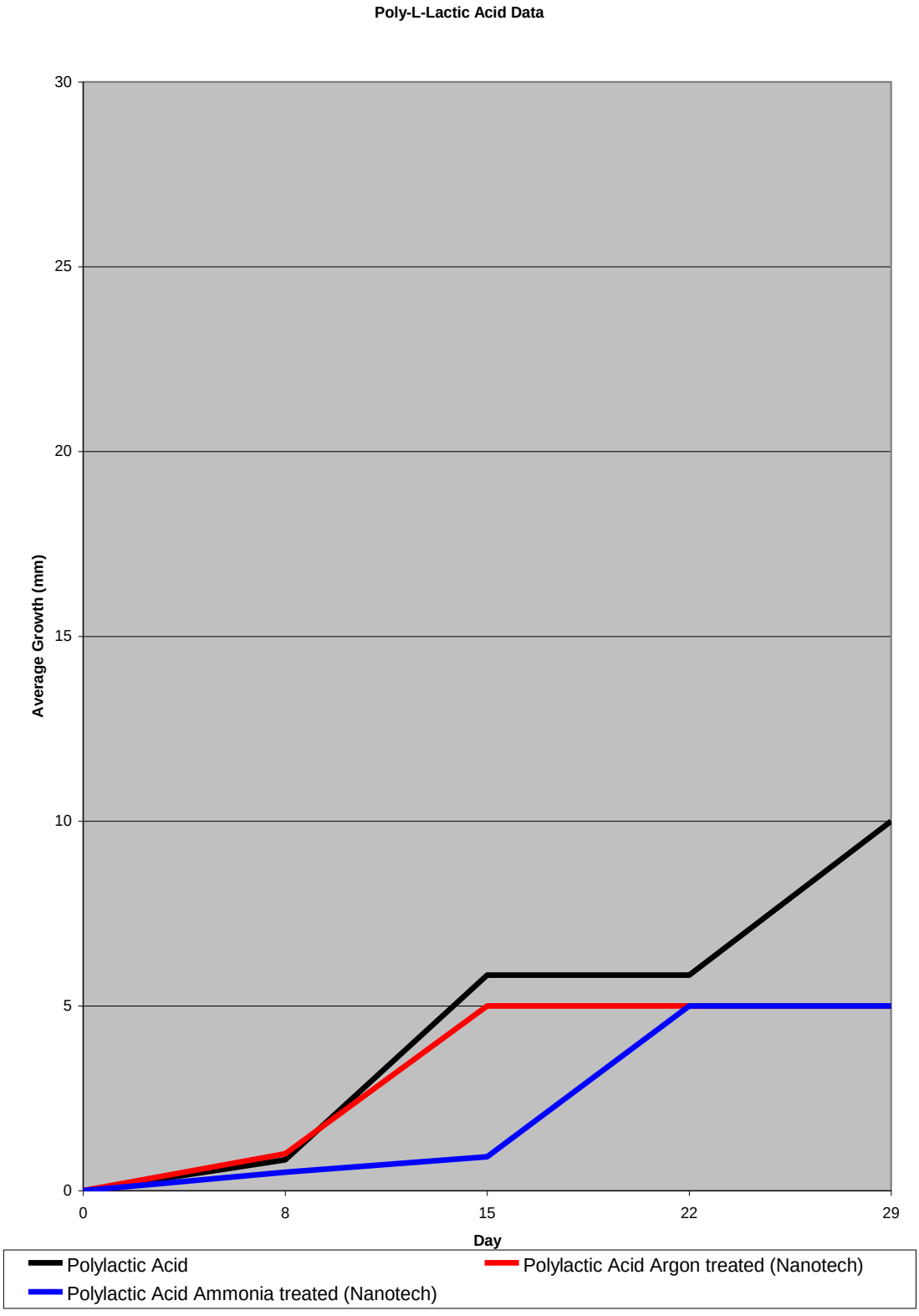


Fig. 4.72 - Graph of average growth of fibroblasts on the polylactic acid samples.

Fig. 4.72 shows the growth of MRC-5 cells along the poly-l-lactic acid samples. The data also shows the control performing better than the plasma treated samples and

therefore, like the polyester, these plasma treatments are not stimulating tissue growth.

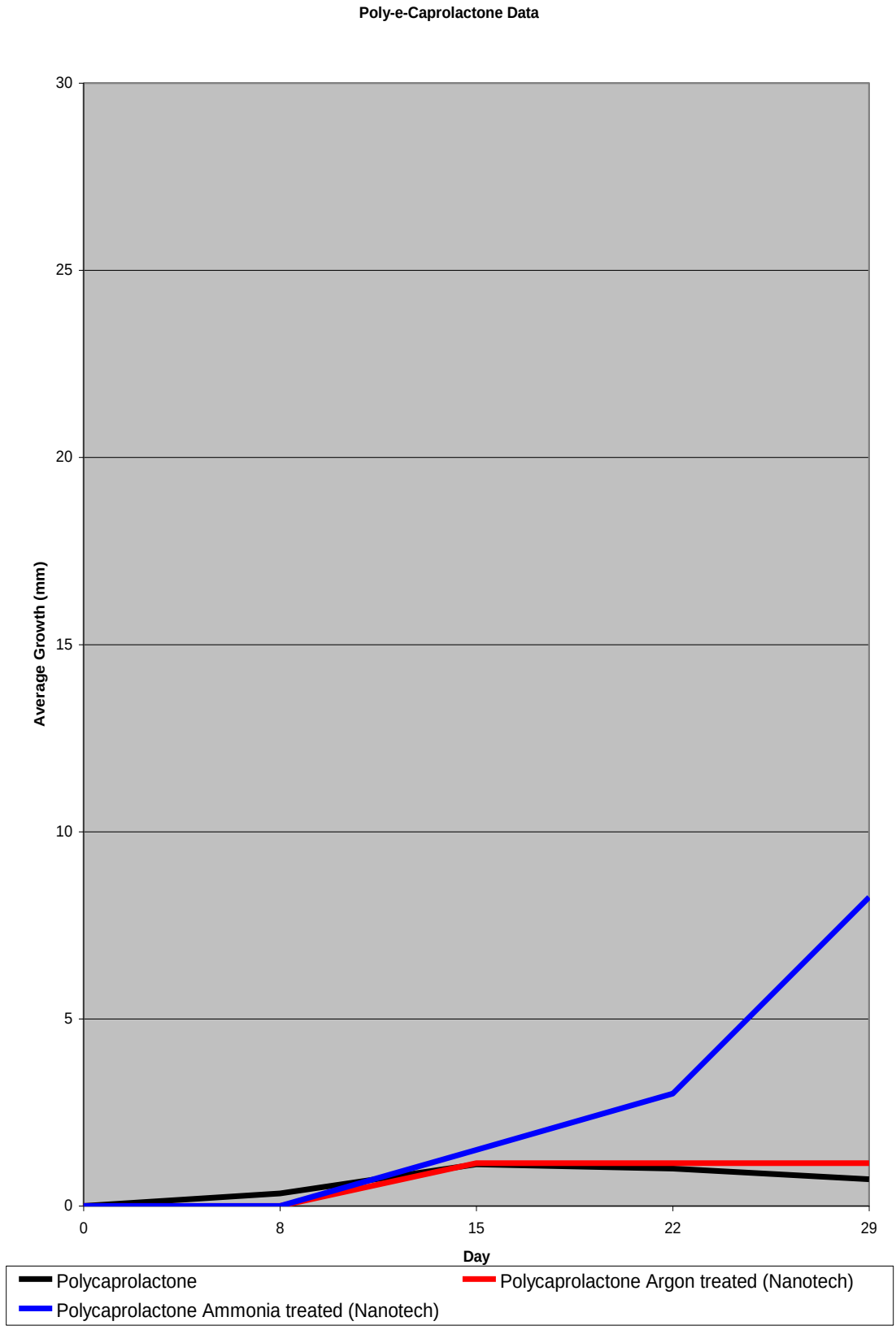


Fig. 4.73 - Graph of average growth of fibroblasts on the poly-ε-caprolactone samples.

Fig. 4.73 shows the growth of MRC-5 cells along the polycaprolactone samples. With this collection of samples, they all perform similarly over the first 15 days. It is only in the last 15 days where there is an obvious difference in growth. The order of ammonia plasma, argon plasma then control can be seen and while the ammonia performs best, the argon plasma treatment is only marginally better than the control. It can also be seen that the argon plasma does not appear to encourage further growth after 15 days, suggesting that the cells are having difficulty growing on this substrate, and are performing poorly on the control which indicates a decline.

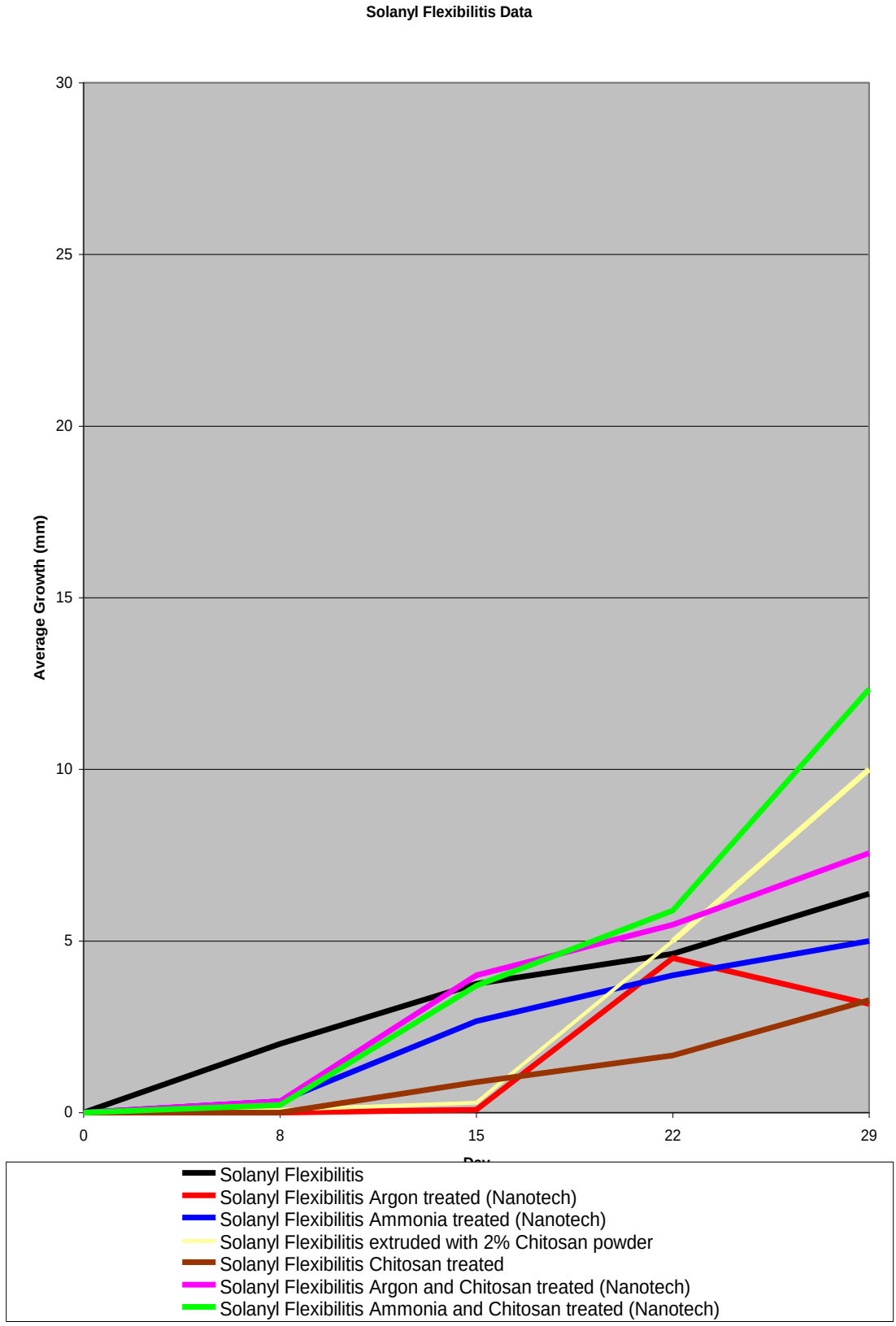


Fig. 4.74 - Graph of average growth of fibroblasts on the Solanyl samples.

Fig. 4.74 shows the growth of MRC-5 cells along the Solanyl samples. This sample collection contains a few variations, so the standard three materials shall be looked at first.

Over the first 15 days, the control and the ammonia plasma samples show steady growth (the ammonia picks up after day 8) but it takes until day 15 for the argon plasma samples to show growth. Over the next 15 days, the control and ammonia plasma samples show continued and steady growth but the argon plasma samples show a burst of growth then a decline. These three samples perform similarly to the PLA samples, with growth around the 5mm point and like the PLA, the two basic plasma treatments do not perform as well as the control. The main difference between the Solanyl control and the PLA is that the PLA control performed better.

The additional treatments with the exception of Solanyl with chitosan coating (no plasma) promote better tissue growth than the control. The Solanyl with argon plasma and chitosan coating and the Solanyl with ammonia plasma and chitosan coating perform similarly except for the last seven days, where the ammonia and chitosan samples show a sharp increase in cell growth. The Solanyl with 2% chitosan does not show noticeable growth for the first 15 days, and then it shows a sharp increase in growth for the last 15 days. This is an interesting finding and could be related to the fact that the chitosan is embedded in the polymer. The sharp increase in growth may occur as the surface of the polymer erodes slightly, exposing the chitosan to the cells.

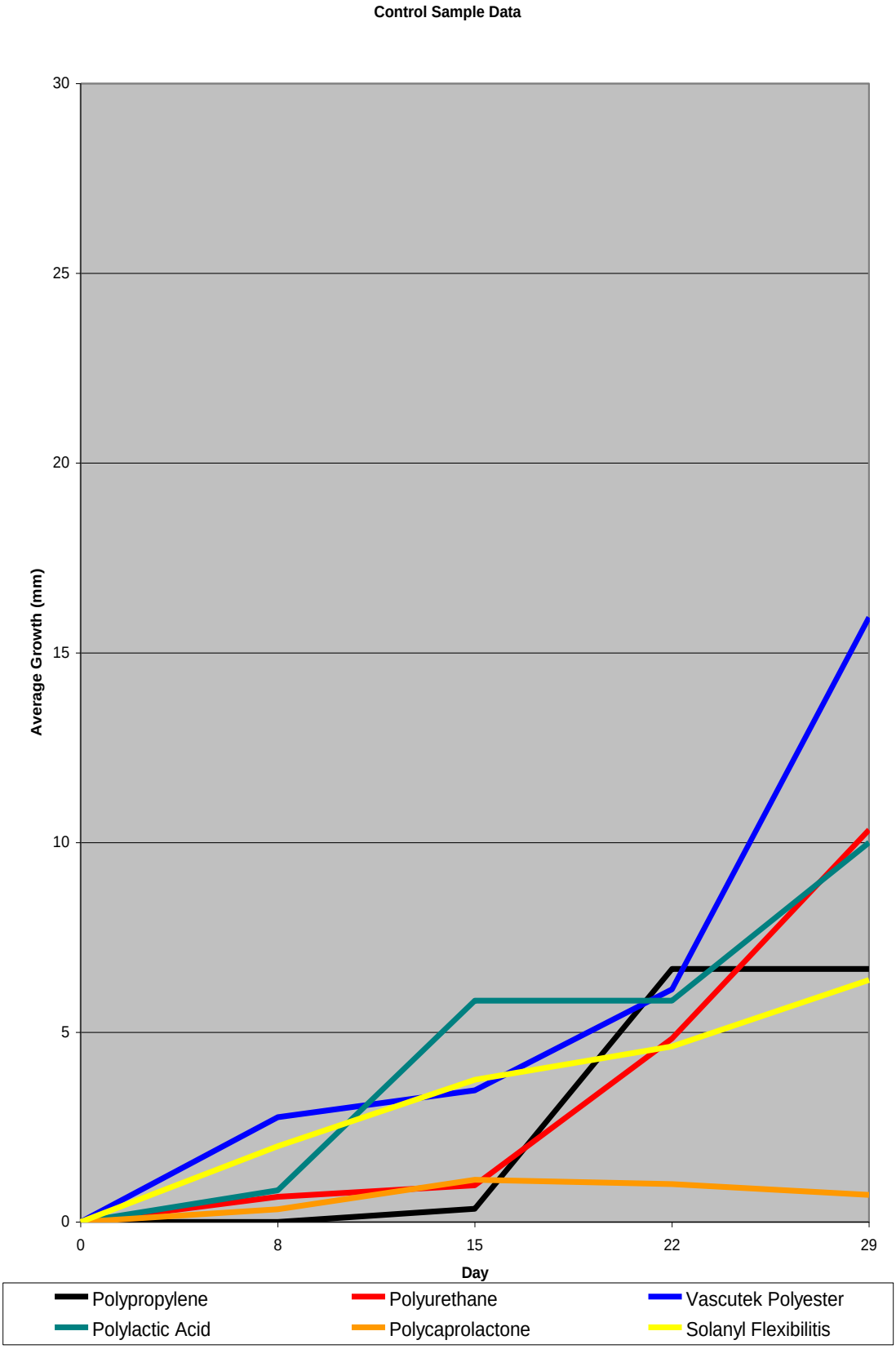


Fig. 4.75 - Graph of average growth of fibroblasts on the control samples.

Fig 4.75 shows the average growth of the control materials over 29 days. Over the first 15 days, there are three distinct groups. The first group showing very little growth includes polypropylene, polyurethane and polycaprolactone. The second group with growth average growth around 4mm includes Vascutek polyester and Solanyl. The last sample is the only sample with growth above 5mm in the first 15 days is the PLA. At day 22, all but the polycaprolactone, group around the 5mm of growth, but over the last 7 days, the growth changes for most of the samples. The polycaprolactone shows no growth. The Solanyl continues to encourage steady growth. The polypropylene shows no further growth after day 22. The polyurethane and polylactic acid produce better growth and the Vascutek polyester samples encourage a steady increase in growth rate indicating that it is the best standard material for supporting tissue growth. This is not a surprising finding as this material is commercially available but also because it is the only material with a knitted structure.

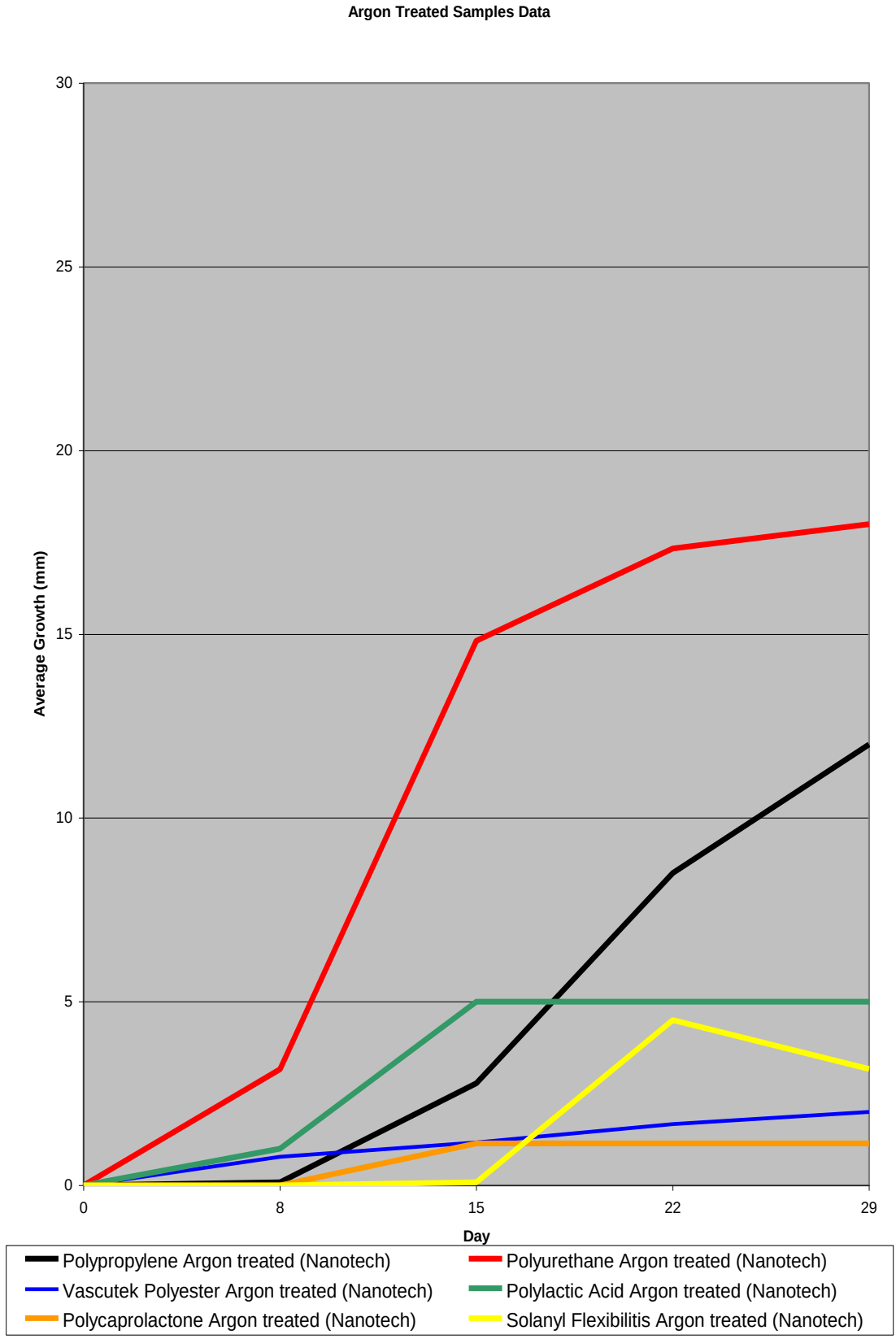


Fig. 4.76 - Graph of average growth of fibroblasts on the argon plasma treated samples.

Fig 4.76 shows the argon treated samples. In relation to fig 4.75, it shows that the argon treatment of polyurethane and polypropylene were a success, as they supported greater growth than the best control material. It also shows how the argon treatment reduced growth compared to the controls, with the exception of polycaprolactone, where the average growth is approximately the same.

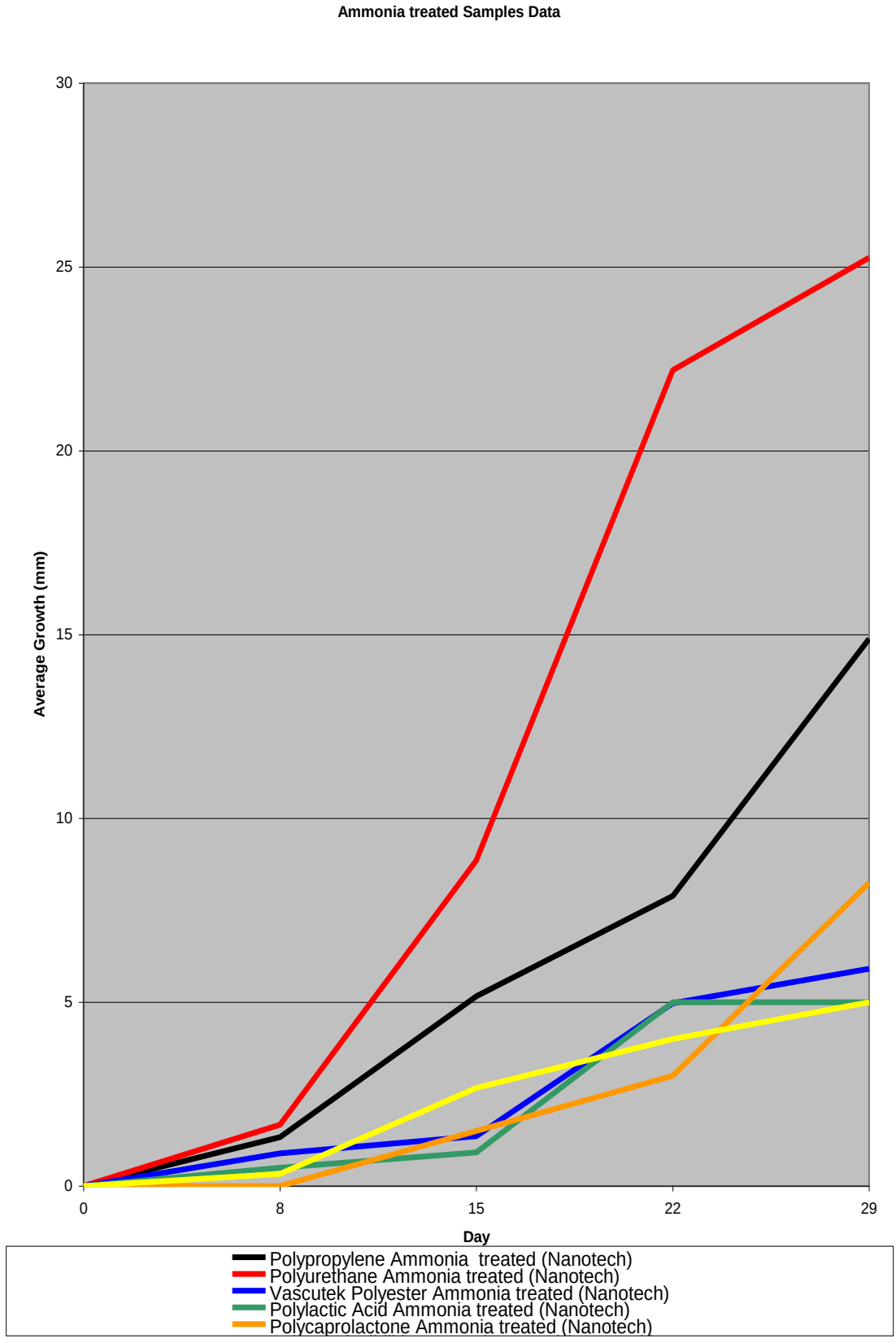


Fig. 4.77 - Graph of average growth of fibroblasts on the ammonia plasma treated samples.

Fig 4.77 primarily shows that the ammonia plasma was the best treatment for the polypropylene and polyurethane. The average growth supported on the polypropylene is almost as much as the average growth on the control Vascutek polyester. This is double the average growth measured on the polypropylene control.

The ammonia treated polyurethane supports the highest average cell growth of all the samples tested, improving the average growth by around 15mm over the polyurethane control, and almost 10mm over the Vascutek polyester control.

Sample	Average growth After 29 Days (mm)	Standard Deviation
Polyurethane Ammonia treated	25.3	8.8
Polyurethane Argon treated	18.0	14.5
Vascutek Polyester	15.9	12.7
Polypropylene Ammonia treated	14.9	14.4
Solanyl Flexibilitis Ammonia and Chitosan treated	12.3	14.9
Polypropylene Argon treated	12.0	14.4
Polyurethane Oxygen treated in (Europlasma)	11.3	14.5
Polyurethane	10.3	12.9
Solanyl Flexibilitis extruded with 2% Chitosan powder	10.0	15.5
Poly-l-lactic acid	10.0	15.0
Polypropylene Chitosan treated	9.6	13.0
Polyurethane with Chitosan powder prior to argon treatment	8.4	13.0
Poly-ε-caprolactone Ammonia treated	8.3	13.6
Solanyl Flexibilitis Argon and Chitosan treated	7.6	11.9
Polyurethane Argon treated in (Europlasma)	7.3	13.0
Polypropylene	6.7	13.2
Solanyl Flexibilitis	6.4	12.0
Polypropylene Ammonia and Chitosan treated	6.2	11.0
Vascutek Polyester Ammonia treated	5.9	9.8
Solanyl Flexibilitis Ammonia treated	5.0	12.2
Poly-l-lactic acid Argon treated	5.0	12.2
Poly-l-lactic acid Ammonia treated	5.0	12.2
Polypropylene Argon and Chitosan treated	3.6	9.9
Solanyl Flexibilitis Chitosan treated	3.3	6.3
Solanyl Flexibilitis Argon treated	3.2	7.8
Vascutek Polyester Argon treated	2.0	3.2
Poly-ε-caprolactone Argon treated	1.1	3.0
Poly-ε-caprolactone	0.7	2.0

Table 4.78 - Average cell growth of samples after 29 days arranged in descending order

As can be seen by the tissue culture data, ammonia treated polypropylene is the fourth best material, with untreated Vascutek polyester performing marginally better.

The top two performers are the plasma treated polyurethanes. The ammonia treated polyurethane comes out top, with an average growth figure 7mm greater than the argon treated polyurethane.

When looking the data, one can see a trend where the ammonia plasma treatment out performs argon treatment, with the exception of the PLA where they both perform the same. This was as hypothesised, as the ammonia will present nitrogen and hydrogen on the surface, much like proteins.

Samples were examined on day 30 using a Leica confocal microscope. Cells were stained using an Invitrogen live/ dead cytotoxicity test (containing ethidium homodimer and calcein AM cellular stains). These images were to be used primarily as conformation of optical microscope measurements and to gain an insight into the quality of cell growth. The fluorescence images were not consistent across the selection of samples and some of them indicated that the cells had been ripped off during the preparation of the samples for fluorescence microscopy.

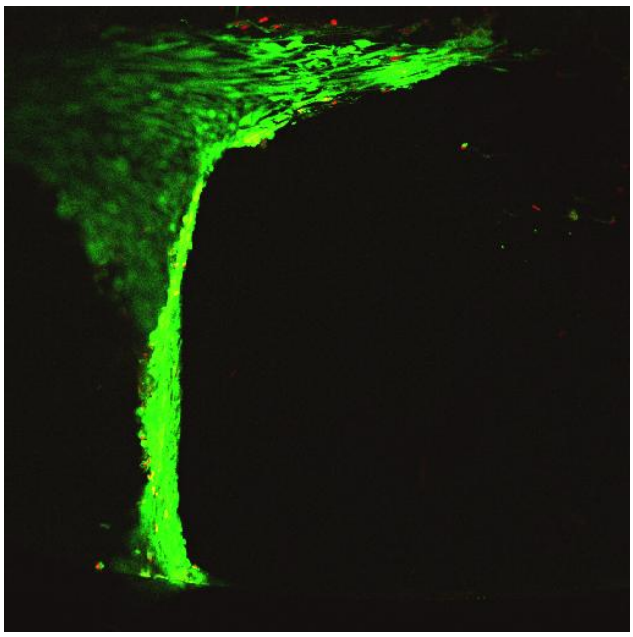


Fig 4.79 Polypropylene 2 sample from 24/09/2005 illustrating an abrupt termination of cells due to the cells ripping off the sample during sample preparation.

The data from the fluorescence imaging can be found in appendix C.2.

Chapter 5 – Discussion

This project aimed to;

- review the advantages and disadvantages of materials used in soft tissue repair and to review potentially alternative materials.
- investigate the reported benefits of using chitosan in relation to medical device applications.
- investigate in depth a limited selection of alternative materials.
- investigate the value of gas plasma treatment on the ability of these materials to support tissue growth in vitro.

This research set out to investigate the current state of biomaterials used for soft tissue repair. Current mesh prostheses made of polypropylene (PP), polyethylene terephthalate (PET) or polytetrafluoroethylene (PTFE) have proven themselves invaluable for the repair of soft tissue defects but they can often lead to complications such as restriction of the abdominal wall mobility, intra-abdominal adhesions with erosion of adjacent organs (or consecutive fistula formation) and inflammatory foreign body reaction where the prosthesis is embedded into a fibrous scar plate causing shrinkage of the mesh area (~40%). They can also provide a surface which bacteria can colonise causing persistent infections that can sometimes only be cleared by the removal of the prosthesis. Polypropylene meshes, which have been in use since 1962, are still the most common material for hernia repair (Morris-Stiff and Hughes, 1998) due to their perceived long term maintenance of tensile strength and low tissue reactivity although in a study conducted in 1998 a failure rate of 10% was recorded (4 out of 40 patients in a single unit) and therefore it was concluded that the complications associated with polypropylene meshes are under reported. While these complications are rarely life threatening, they highlight the need for further research into these devices. Degradable biomaterials used (e.g. polyglycolic acid) can also cause complications such as the recurrence of the hernia due to failure of the device and inflammatory reactions caused by rapid degradation of the material.

It is hypothesised that complications associated with medical devices are associated with an inability to assimilate with the host tissue, therefore by improving host tissue regeneration, complications will be reduced.

To address the issue of prosthesis related infections, there needs to be a material that could inhibit bacterial growth that was suitable for use within the human body. Chitosan (& chitin) have been reported to inhibit bacterial growth and fungal growth whilst enhancing human tissue growth (and many other attributes). It was therefore hypothesised that by using chitosan as a coating or incorporating chitosan into a biomaterial, tissue regeneration would be enhanced and prosthesis related infections would be reduced.

From these two hypothesises two lines of research became evident.

- To examine the efficacy of chitosan as a bacteriostat.
- To examine a range of biomaterials for their ability to encourage fibroblast growth and see if fibroblast growth could be improved by modifying the surface of the biomaterial.

5.1 Examination of Chitosan as a Bacteriostat

As a result of the reports of chitosan as a bacteriostat in the literature, this study was devised to determine the efficacy of chitosan against a common and prolific opportunistic pathogen. Staphylococcus epidermis was also included in the early study but MRSA was the most relevant candidate for testing the efficacy of chitosan. MRSA is gram positive and resistant to certain antibiotics. It is also one of the most problematic infections to clear when acquired in a hospital environment and can be life threatening and therefore it is the most interesting bacterium to test the bacteriostatic claims reported for chitosan.

The means of testing the bacteriostatic effect of chitosan followed two distinct paths. One was to examine the ability of chitosan to inhibit growth on a surface and the

other was designed to determine a quantifiable effect on MRSA (and to a lesser extent S. *epidermis*) in broth culture by varying the quantity of chitosan in the broth. The methods employed were of an evolutionary nature in that deficiencies in one method were addressed in the next.

Method 1 - Chitosan dissolved in dilute acetic acid incorporated into nutrient agar Vs MRSA 9551 and Staphylococcus epidermis.

Method 1 was the beginning of the method development where initial investigations into both aspects of this study started producing preliminary data but the methods diverged as the requirements of each method were developed. From there, method 3 examined the inhibition of growth around chitosan film or materials coated in chitosan film while methods 2 and 4 examined the growth of MRSA in solutions containing chitosan.

Method 2 - Chitosan dissolved in acetic acid added to nutrient broth Vs MRSA 9551 and Staphylococcus epidermis

The data for Method 2 suggested a bacteriostatic relationship between chitosan and MRSA. This experiment illustrates the bacteriostatic effect of chitosan against MRSA (although the data is less clear for S.*epidermis*). This experiment shows the first four hours of bacterial growth in the presence of chitosan in detail, although after the first 4 hours, there is a 22 hour gap between measurements. The data clearly shows the lag phase and logarithmic growth phase but not the stationary phase or death phase. This experiment may have benefited from hourly measurements over the 26 hour period, but with the resources available, this was not possible.

Method 3 - Testing of Chitosan treatment of Cotton cloth

Method 3 was designed to expand on the early investigation in method 1 of chitosan film. The cotton cloth coated with the chitosan solution demonstrated an observable zone of inhibition. The polypropylene coated with chitosan solution was not so effective. The result for polypropylene coated in chitosan against MRSA produced a

partial zone of inhibition. When tested with S. *epidermis*, no zone of inhibition was observed.

Method 3 examined the effect of a chitosan coated material on an inoculated agar plate. Although the chitosan coating was not pH neutralised, the results indicate a zone of inhibition around most of the treated samples. These methods indicate that chitosan coating may be a viable means of producing materials which inhibit bacterial growth with the added advantage that it is easy to apply. This application could be used in both medical devices and on textiles used within the hospital environment although further research to optimise this coating is recommended.

Method 4 - Chitosan suspended in nutrient broth Vs MRSA

Method 4b was the final evolution of methods 2 and 4. This method yielded a large quantity of data on the efficacy of chitosan in suspension Vs MRSA. These results suggest that the bacteriostatic effect varies with the quantity of chitosan presented to the bacteria but it does not appear, from this data, to be a direct relationship.

The studies in method 4b suggest that there is a large variation in the efficacy of chitosan powder as a bacteriostat against MRSA. There are two potential explanations for the results in the 06/10/03 and 19/01/05 studies. First, the efficacy of chitosan may be related to the concentration of bacteria. Second, the initial measurements were taken after 24 hours, and it is possible that the control may have already finished the exponential phase and stationary phase and started on the death phase before the first measurements were taken. In future, it would be interesting to measure the samples every 4 hours in the first 24 hours to see if the extra resolution proves this hypothesis to be the case.

Examination of Chitosan as a Bacteriostat Summary

The chitosan study set out to examine the efficacy of chitosan as a bacteriostat. To that end, it has been observed that chitosan does have a bacteriostatic effect. In film

form the chitosan produced a clear zone of inhibition against MRSA and in suspension it caused inhibition of growth for the majority of samples tested. In terms of producing quantitative data, the methods developed towards the end of the study could still not elucidate a relationship between the quantity of chitosan suspension and the effect on MRSA.

This study indicates that when chitosan is applied to a material as a film, it has a bacteriostatic effect against MRSA and when it is used as a suspension it can produce a bacteriostatic effect although it is a variable one. As the main application of chitosan in this project is as a coating, this data suggests it will impart bacteriostatic properties to the material it is applied to.

It has been reported that among other properties that chitosan is an effective bacteriostat, but this study indicated the need for further studies to clarify the susceptibility of a wide range of pathogens to chitosan. Chitosan is a difficult material to study in these experiments as it is not readily soluble except in acidic solutions and is therefore difficult to study a neutral environment. Chitosan has an effect on bacteria as reported in the literature, but the results of this study found that the results varied greatly depending on how the chitosan was presented and the concentration of the inoculum.

There are many sources of chitosan including crustacea and fungi and many different degrees of deacetylation (chitosan is stated as being greater than 70% deacetylated). There are also many modifications of chitosan, including water soluble chitosan but this study used the same chitosan throughout this study and the tissue culture study (high molecular weight, high purity squid chitosan). Chitosan derived from different organisms and different deacetylation techniques may demonstrate different levels of bacterial growth inhibition. With all of the reported benefits of using chitosan, research will continue and further applications will be developed. Until experimental analysis reveals a better chitosan for inhibiting bacterial growth, the high purity squid chitosan will be useful as a bacteriostatic coating for medical prostheses to inhibit post-operative infections.

5.2 Production of Biomaterial Samples

The biomaterial samples used in this research were produced using a variety of production methods. This variety of production methods introduced differences in morphology of the biomaterials. This factor limits the conclusions that can be drawn between biopolymers used in this research although with these differences noted, careful comparisons may be made. The two samples with the greatest difference in surface morphology were the PLA and the polyester. The polyester and PLA were included because they were so different from the extruded samples and because they were expected to outperform the extruded samples due to increased cell adhesion.

5.3 Sample Characterisation

5.3.1 Differential scanning calorimetry (DSC) Analysis

The DSC analysis provided the melting points of the polymers. Due to the low melting point of the poly-ε-caprolactone, the samples could not be sterilised using an autoclave (121ºC for 15 minutes) so ethylene gas sterilisation was used instead.

5.2.2 SEM Analysis

The SEM analysis of the samples showed a degree of variation in the surfaces of the different polymers. Some of the polymers had a very smooth surface (Solanyl and polyurethane), some had minor grooving from the extrusion process (polypropylene and poly-ε-caprolactone) and two materials had very different surfaces (polyester and polylactic acid). These differences affect the growth of cells on the materials therefore conclusions from the tissue culture study should only be made with these differences in mind. Plasma treatment made no observable change to the surfaces of the biomaterials, but as seen in fig. 4.35 and 4.36, plasma treatment has a great effect on the hydrophilicity/ hydrophobicity of a polymer.

Looking at the Solanyl containing 2% chitosan (w/w), particles of chitosan powder can be seen on the surface of the sample. It appears from fig. 4.52 that the chitosan powder was not evenly distributed through the Solanyl. This should not be of great

importance for this study, but should be addressed if this material is to be developed further.

The PLA images illustrate the porous nature of the film cast using DCM evaporation. The pore sizes were measured and compared to the area of the images used in the pore size measurements.

Average area of pores for the three images (μm^2)	8.90
Average percentage of pores for the three images	47.22%

Table 5.1 summary of the pore size measurements.

The PLA shows a highly porous structure although the average pore size and percentage porosity are both lower than the optimal sizes/ percentages quoted by Oh (186–200 μm pore size (Oh et al., 2007)) and Minns (10-50 μm pore size overall porosity of 85-90% (Minns, 1999)).

5.4 Tissue culture study

This project aimed to determine which biomaterial or surface treatment yielded the greatest fibroblast growth and to test a selection of surface treatments to see if they can be used to improve current biomaterials. The theory behind this was that by testing biomaterials for their ability to support fibroblast growth, a logical foundation is created for the design and optimisation of soft tissue repair prostheses. This was achieved by inoculating the biomaterial samples at one end with a 20µl drop of MRC-5 cells and measuring their growth along the 30mm strip of material. As the samples could not all be tested at once, the samples were tested in mixed groups of three treatments of three materials in triplicate and each sample was tested 9 times. Therefore 243 samples were tested in total (27 different samples tested 9 times each), each tested over a 29 day period.

Prior to testing the biomaterials with MRC-5 cells, the materials were washed in 70% ethanol, ethylene gas sterilised and examined using SEM to observe any differences between the materials and to observe any differences in the surfaces as a result of modification. When examining the data in chapter 4.1, the surface topography can be seen. By examining these SEM images, it can be seen that the surface topography is different for each material. The difference is modest between polypropylene, Tuftane and Solanyl and polycaprolactone. With the PLA and polyester samples the surface topography is quite different. This would have an effect on how well the MRC-5 cells grew on the substrate therefore comparisons should be made only with these differences in mind. No difference was observed in the SEM images between the plasma treated samples and the standard materials, although the Solanyl containing 2% chitosan had slight bumps on the surface due to the incorporation of the chitosan powder.

The tissue culture study performed well as a comparison between the six different materials and produced useful data on how the plasma treatment affected tissue growth on the different materials. In addition to evaluating plasma treatment,

selections of chitosan based treatments were tested but these were primarily explorative in their nature (to examine the viability of such treatments).

Polypropylene, the most commonly used biomaterial in surgery, made a useful reference point for studying biomaterials. It was also the starting point for examining modifications that can enhance biocompatibility. Three treatments were shown to improve fibroblast growth on polypropylene (argon plasma, ammonia plasma and chitosan coating with no prior plasma treatment). Argon and chitosan treated polypropylene and ammonia and chitosan treated polypropylene both demonstrate inferior tissue growth compared to the control after 29 days, although growth is faster over the first 15 days, therefore any benefit derived from these treatments is transitory.

The polyurethane data indicates that three treatments produced enhanced growth over the unmodified material, the ammonia treatment, the argon treatment and the oxygen treatment, although all of the treatments show enhanced growth over the first 15 days. This reflects what is seen for the polypropylene data. The two samples of polyurethane treated with argon plasma on different plasma treatment equipment produce radically different results. The polyurethane treated with argon on the Europlasma equipment and the polyurethane treated with argon on the Nanotech equipment were used to illustrate that although the equipment was different, the effect was the same but as can be seen by the data, they produced quite different results. This was quite unexpected and will need to be investigated further. Shortly after treating the polyurethane with argon and oxygen on the Europlasma equipment, the plasma equipment became faulty, so other samples treated on the Europlasma equipment were discarded and the plasma treatment was performed in the older Nanotech equipment. Although the Europlasma argon and ammonia treatments performed poorly compared to the equivalent Nanotech samples, the oxygen plasma treated sample showed improved growth over the standard material. This suggests that if the sample was treated with oxygen plasma on the Nanotech equipment, the oxygen plasma may have performed very well. Oxygen plasma could not be

produced on the Nanotech equipment as there was no oxygen gas available at the time of plasma treatment.

The Vascutek polyester data clearly shows that argon and ammonia plasma treatment does not improve fibroblast growth. What is interesting about the data is that the ammonia plasma still outperforms the argon plasma.

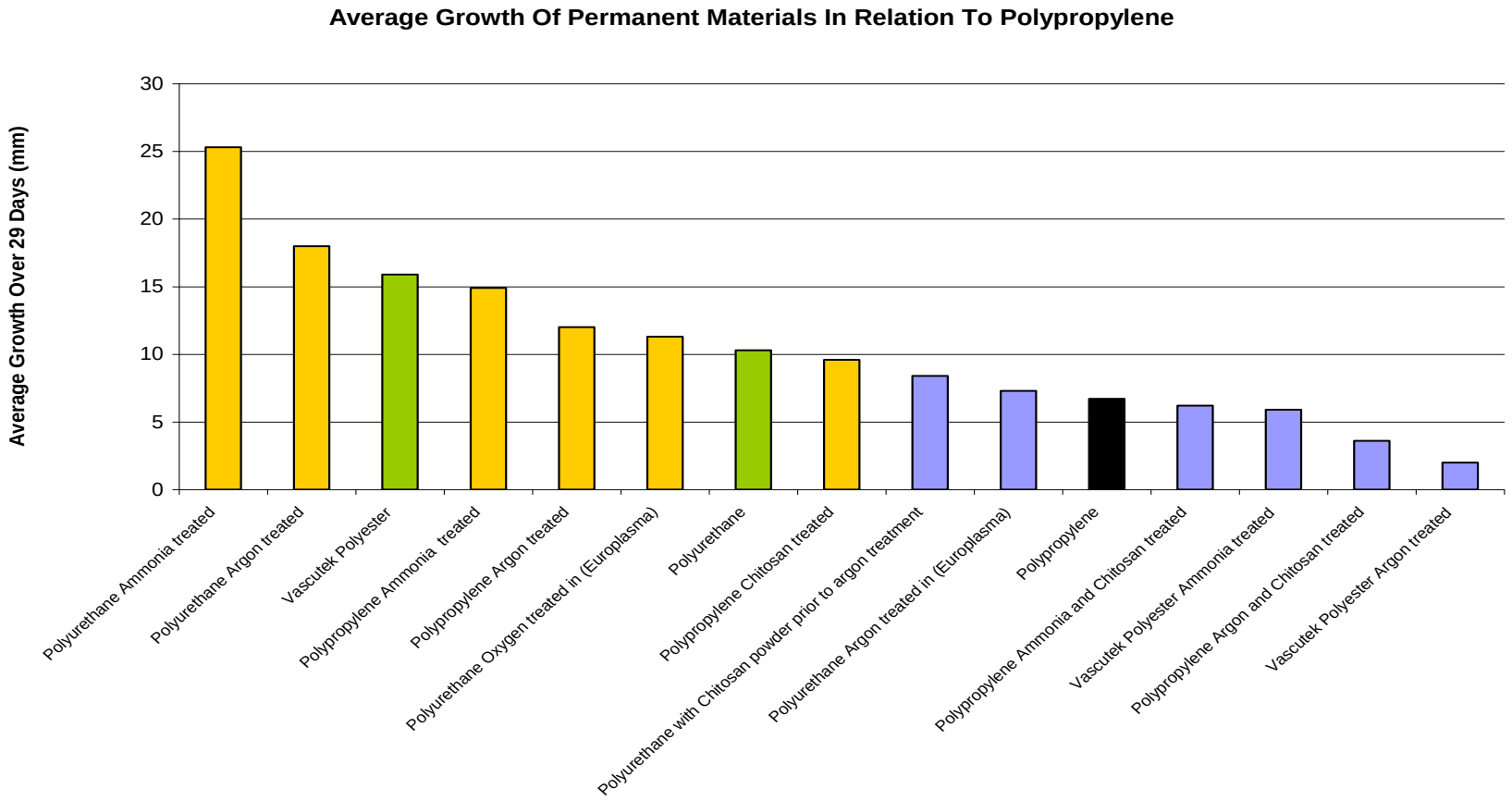
The PLA was predicted to perform well, considering it had a porous surface. It performed better than untreated polypropylene, untreated poly-ε-caprolactone and untreated Solanyl. The untreated PLA does not promote as much growth as the untreated Vascutek polyester, although it does outperform the untreated polypropylene. None of the plasma treatments improved growth on the PLA.

The untreated poly-ε-caprolactone performed poorly. Argon treatment had little effect compared to the control. Ammonia treatment promoted approximately thirteen times the growth of the poly-ε-caprolactone control. This is the greatest improvement over the control recorded. In addition, ammonia treatment of poly-ε-caprolactone promoted growth slightly greater than the polypropylene control.

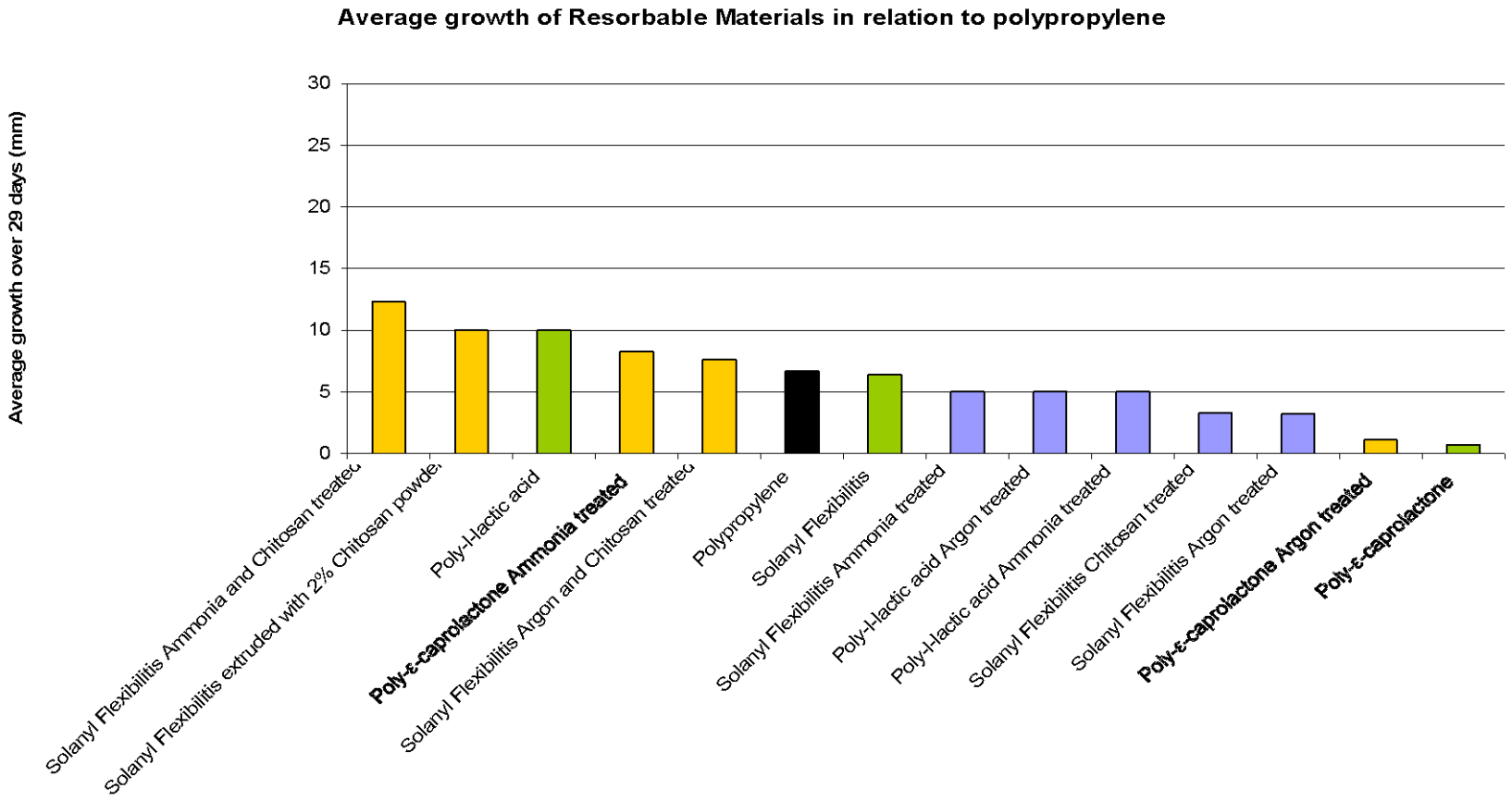
The data for Solanyl indicated that the argon, ammonia and chitosan treatment (without plasma pre-treatment) produce products that are inferior to the control material. In contrast to the polypropylene samples, the argon and ammonia pre-treated Solanyl coated in chitosan both perform better than the control material, with the ammonia and chitosan treated Solanyl promoting twice the growth of the control. The Solanyl containing 2% chitosan performed well; therefore this method of incorporating chitosan in degradable biomaterials requires further study to determine the best ratio of chitosan to polymer and to examine which other degradable biopolymers can benefit from the addition of chitosan.

The data for the control samples reveals that three of the tested materials support greater fibroblast growth than polypropylene (Vascutek polyester, polyurethane and

poly lactic acid) but it is clear that plasma treatment can be used to produce a better surface for fibroblast growth. With the plasma treatment, the samples were considered a success if they supported cell growth greater than the untreated material. They were a greater success if they encouraged cell growth beyond the untreated material and produced growth greater than polypropylene (the benchmark).



Graph 5.2 - Summary of fibroblast growth on permanent materials. Polypropylene is shown in black as it is the control. The untreated materials are shown in green. The yellow bars are samples where the treatment improved fibroblast growth over the untreated material. Blue bars are samples where the surface treatments reduced fibroblast growth compared to the untreated samples.



Graph 5.3 - Summary of fibroblast growth on resorbable materials. Polypropylene is shown in black as it is the control. The untreated materials are shown in green. The yellow bars are samples where the treatment improved fibroblast growth over the untreated material. Blue bars are samples where the surface treatments reduced fibroblast growth compared to the untreated samples.

The argon and ammonia treated polyurethane were clearly the most successful treatments, but growth on ammonia plasma treated polypropylene was more than twice the growth of standard polypropylene. Ammonia and chitosan treated Solanyl produced the best growth on a degradable material, although there is only 2.3mm difference in average growth between this and Solanyl containing 2% chitosan which would make both materials viable options for degradable prostheses. The Ammonia and chitosan treated Solanyl would also impart a bacteriostatic effect due to the chitosan film therefore inhibiting prosthesis related infections. The Solanyl containing 2% chitosan may also impart this protection, although further study would be required to prove this. The oxygen plasma treated polyurethane (Europlasma) does not perform as well as the argon plasma treated polyurethane (Nanotech) or the ammonia plasma treated polyurethane (Nanotech, but when the poor performance of the Europlasma treated argon sample is taken into account (an average growth of 10.7 difference between the Europlasma and Nanotech argon treated samples) it can be suggested that oxygen plasma could perform better if produced on the Nanotech equipment.

Conclusions

It is clear from the data that chitosan is affecting the growth of MRSA, with chitosan film producing observable zones of inhibition against MRSA, although there is not a direct relationship between the quantity of chitosan powder in solution and the effect on growth. This project achieved its aim to examine the bacteriostatic effect but further work will be required to find a direct relationship. Medical applications may include implanted devices and textiles used in the hospital environment (e.g. soft furnishings, nurses' uniforms and doctors' coats).

The tissue culture study completed the objective of comparing a range of biomaterials and surface treatments in a consistent and unbiased manner, producing interesting results. It is also clear from the data that gas plasma treatment can improve fibroblast growth on some of the biomaterials.

Ammonia and chitosan treated Solanyl and Solanyl containing 2% chitosan proved to be the best degradable biomaterials tested. These materials should be tested *in vivo* for their ability to repair soft tissue defects. The ammonia and chitosan treated Solanyl may also be tested as repair prostheses for non-sterile tissue repair, perhaps as a suture material as it should inhibit infections associated with such wound closures. If the Solanyl containing 2% chitosan proves to be effective as a bacteriostat, it too may be suitable for this application.

The polyurethane sample treated with ammonia plasma appears to be an interesting candidate for further study as the only material with 100% survival of cells in culture and the best growth measurements over the 29 days. This material requires further study to determine its efficacy in vivo and to develop the best design to support the load of abdominal and pelvic floor contents. The next stage for this material is to design mesh prosthesis for animal trials, so the efficacy can be determined in vivo.

Other materials performed well, with ammonia treated polypropylene yielding a great improvement in cell growth over untreated polypropylene. As polypropylene is already a widely used material for soft tissue repair, it would be feasible to produce a new polypropylene prosthesis with ammonia treatment. This would provide a prosthesis with the handling and mechanical characteristics that surgeons are familiar with but with the benefit of improved incorporation within the patient.

As hypothesised, the ammonia plasma consistently performs better than the argon plasma (with the exception of polylactic acid, where it performs the same as argon). Further work will be needed to determine if the ammonia does deposit NH groups as hypothesised.

Further study is required into the use of gas plasma, considering the difference in results from polyurethane samples treated with the same gas using different equipment. With further testing and optimisation, the Europlasma equipment could produce results equivalent to the Nanotech equipment by altering the gap between the charged plates. The Europlasma has advantages over the Nanotech equipment in that it is computer controlled and should therefore be able to produce more consistent results.

Recommendations for further study of gas plasma include;

- Examining a greater range of gases for their ability to enhance cell proliferation on biomaterials.
- Examine the efficacy of gas plasma treatments for a broader range of biomaterials gases for their ability to enhance cell proliferation on biomaterials.
- Examining different parameters within the plasma chamber to optimise gas plasma treatments for enhancing cell proliferation on biomaterials.
- Examine the efficacy of atmospheric plasma treatment as an alternative to low pressure plasma treatment for coating biomaterials

Recommendations for further developing the methods used to examine chitosan as a bacteriostat include;

- Measuring the growth of the bacteria in the presence of chitosan (in suspension) at one to two hour periods during the first 24 to 48 hours.
- Testing the bacteriostatic effect of chitosan against a greater range of pathogens.
- An investigation into the molecular basis behind the bacteriostatic effect so it can be optimised and then verified.
- Testing the efficacy of chitosan derived from different sources, with different molecular weights to determine which chitosan has the greatest effect on bacterial growth.

Recommendations for developing optimal soft tissue repair prosthesis include an *in vivo* study, where the response of the immune system can be taken into account. This would include testing the selected biomaterials in a range of morphologies as well as a range of treatments as the morphology of the implant will have a great effect on the response of host tissue to the medical device. Novel production techniques may allow the production of materials with biomimetic structures that may enhance biocompatibility.

In conclusion, it is the recommendation of this study that the optimal material tested in this project was ammonia plasma treated polyurethane. The next stage of development would be to develop prototype prosthesis and perform *in vivo* testing to gather data on tissue regeneration and immune response.

Appendix

Items in the appendix consist of supporting material of considerable length or additional data that would interrupt the flow of the thesis.

A. Examination of Chitosan as a Bacteriostat

A.1 Details of media used for the examination of chitosan as a bacteriostat

Nutrient agar (NA)			
	pH	7.4 approx	
	Oxoid code	cm3	
	lot	01036886	
	2.8g per 100mls distilled water		
	formula [per litre]		
	lab-lemco powder	[Oxoid L29]	1g
	Yeast extract	[Oxoid L20]	2g
	Peptone	[Oxoid L37]	5g
	Sodium chloride		5g
	Agar No 3	[Oxoid L13]	15g

Nutrient broth (NB)			
	pH	7.5 +/- 0.2	
	Oxoid code	cm67	
	lot	10559702	
	2.5g per 100mls distilled water		
	formula [per litre]		
	lab-lemco powder	[Oxoid L29]	10g
	Peptone	[Oxoid L37]	10g
	Sodium chloride		5g

Plate Count Agar (PCA)			
	A medium for the enumeration of viable organisms on milk and dairy products		
	pH	7.0 approx	
	Oxoid code	CM325	
	formula [per litre]		
	Yeast extract	[Oxoid L21]	2.5g
	Tryptone	[Oxoid L42]	5g
	Dextrose		1g
	Agar No1	[Oxoid L11]	9g

Table A.1

A.2 Unedited graph from Method 2c

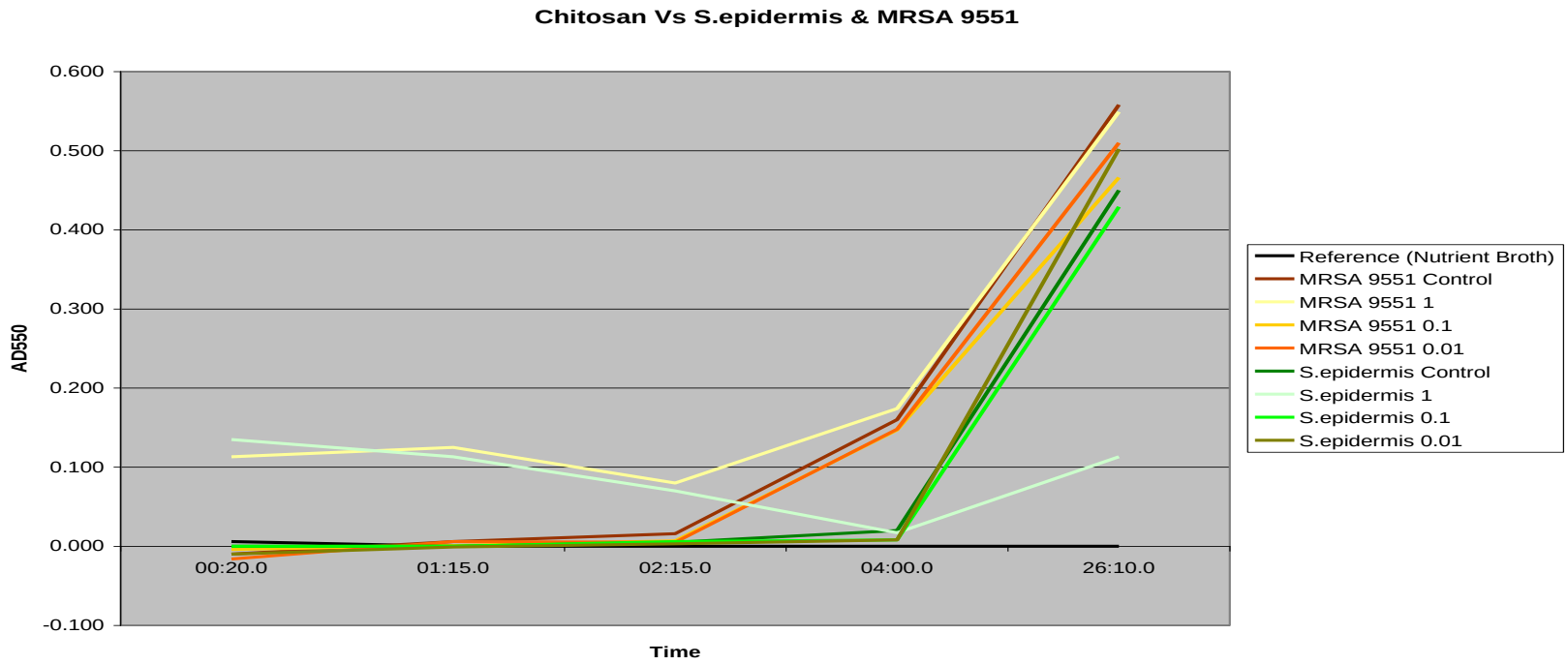


Fig. A.2

Unedited graph for method 2c including 1g chitosan suspension reading.

A.3 Data from Method 4b

Method 4b

07/10/03 Data

Table A.3

Chitosan and MRSA data				06/10/03			
initial inoculum							
cell count mean				48.2			
cells per ml				1.93×10^{8}			
cells in 200µl inoculum				3.86×10^{7}			
Results from 07/10/2003							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values
	10^{-6}	10^{-7}	10^{-8}	10^{-6}	10^{-7}	10^{-8}	
c1							
c2			962			9.62×10^{-10}	
c3		44092			4.41×10^{-11}		
							2.69×10^{-11}
0.02 - 1			3504			3.50×10^{-11}	
0.02 - 2		593	290		5.93×10^{-9}	2.90×10^{-10}	
0.02 - 3			457			4.57×10^{-10}	
							1.08×10^{-11}
0.04 - 1			248			2.48×10^{-10}	
0.04 - 2							
0.04 - 3			1572			1.57×10^{-11}	
							9.10×10^{-10}
0.06 - 1			412			4.12×10^{-10}	

0.06 - 2			29			2.90×10^{-9}	
0.06 - 3							
							2.21×10^{-10}
0.08 - 1			352			3.52×10^{-10}	
0.08 - 2			351			3.51×10^{-10}	
0.08 - 3			649			6.49×10^{-10}	
							4.51×10^{-10}
0.1 - 1							
0.1 - 2			3044			3.04×10^{-11}	
0.1 - 3							
							3.04×10^{-11}
Results from 08/10/2003							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values
	10^{-6}	10^{-7}	10^{-8}	10^{-6}	10^{-7}	10^{-8}	
c1			1016			1.02×10^{-11}	
c2			407			4.07×10^{-10}	
c3			3992			3.99×10^{-11}	
							1.81×10^{-11}
0.02 - 1			3364			3.36×10^{-11}	
0.02 - 2			1616			1.62×10^{-11}	
0.02 - 3			2216			2.22×10^{-11}	
							2.40×10^{-11}
0.04 - 1			305			3.05×10^{-10}	
0.04 - 2			2276			2.28×10^{-11}	
0.04 - 3			105			1.05×10^{-10}	

							8.95×10^{-10}
0.06 - 1			1920			1.92×10^{-11}	
0.06 - 2			1800			1.80×10^{-11}	
0.06 - 3			1788			1.79×10^{-11}	
							1.84×10^{-11}
0.08 - 1			958			9.58×10^{-10}	
0.08 - 2			788			7.88×10^{-10}	
0.08 - 3			3200			3.20×10^{-11}	
							1.65×10^{-11}
0.1 - 1			2340			2.34×10^{-11}	
0.1 - 2			6636			6.64×10^{-11}	
0.1 - 3			2416			2.42×10^{-11}	
							3.80×10^{-11}
Results from 09/10/2003							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values
	10^{-6}	10^{-7}	10^{-8}	10^{-6}	10^{-7}	10^{-8}	
c1			2610			2.61×10^{-11}	
c2			1990			1.99×10^{-11}	
c3			563			5.63×10^{-10}	
							1.72×10^{-11}
0.02 - 1			1610			1.61×10^{-11}	
0.02 - 2			1618			1.62×10^{-11}	
0.02 - 3			1380			1.38×10^{-11}	
							1.54×10^{-11}
0.04 - 1			842			8.42×10^{-10}	

0.04-2			1262			1.26×10^{-11}	
0.04-3			1484			1.48×10^{-11}	
							1.20×10^{-11}
0.06-1			1996			2.00×10^{-11}	
0.06-2			2050			2.05×10^{-11}	
0.06-3			1760			1.76×10^{-11}	
							1.94×10^{-11}
0.08-1			3094			3.09×10^{-11}	
0.08-2			2766			2.77×10^{-11}	
0.08-3			1182			1.18×10^{-11}	
							2.35×10^{-11}
0.1-1			2644			2.64×10^{-11}	
0.1-2						0	
0.1-3			2228			2.23×10^{-11}	
							1.62×10^{-11}

20/10/03 Data

Table A.4

Chitosan and MRSA data	20/10/03		
initial inoculum			
cell count mean	20.6		
cells per ml	8.24×10^{7}		
cells in 200µl inoculum	1.65×10^{7}		
Each culture for this experimental run was plated out two times (a & b)			
Results from 21/10/03			
	cell counts from plate count agars (by	cells per ml for these values (by dilution)	Average of cells per ml values

	dilution)						
	10^{-7}	10^{-8}	10^{-9}	10^{-7}	10^{-8}	10^{-9}	
c1a	1	1		1.00×10^{-7}	1.00×10^{8}		
c1b		3			3.00×10^{8}		
c2a	85	11		8.50×10^{8}	1.10×10^{9}		
c2b	65	6	1	6.50×10^{8}	6.00×10^{8}	1.00×10^{9}	
c3a	60	11	3	6.00×10^{8}	1.10×10^{9}	3.00×10^{9}	
c3b	82	10		8.20×10^{8}	1.00×10^{9}		
							8.56×10^{8}
0.002-1a	32	1		3.20×10^{8}	1.00×10^{8}		
0.002-1b	51	3		5.10×10^{8}	3.00×10^{8}		
0.002-2a	64	4	1	6.40×10^{8}	4.00×10^{8}	1.00×10^{9}	
0.002-2b	68	2	1	6.80×10^{8}	2.00×10^{8}	1.00×10^{9}	
0.002-3a		12	2		1.20×10^{9}	2.00×10^{9}	
0.002-3b							
							6.96×10^{8}
0.004-1a	55	5	2	5.50×10^{8}	5.00×10^{8}	2.00×10^{9}	
0.004-1b	93	6	1	9.30×10^{8}	6.00×10^{8}	1.00×10^{9}	
0.004-2a	104	10		1.04×10^{9}	1.00×10^{9}		
0.004-2b	142	14	2	1.42×10^{9}	1.40×10^{9}	2.00×10^{9}	
0.004-3a	130	8		1.30×10^{9}	8.00×10^{8}		
0.004-3b	118	3		1.18×10^{9}	3.00×10^{8}		
							1.07×10^{9}
0.006-1a	131	21		1.31×10^{9}	2.10×10^{9}		
0.006-1b	92	16		9.20×10^{8}	1.60×10^{9}		
0.006-2a	103	19		1.03×10^{9}	1.90×10^{9}		
0.006-2b	95	11		9.50×10^{8}	1.10×10^{9}		

0.006-3a	71			7.10×10^{8}			
0.006-3b	78			7.80×10^{8}			
							1.24×10^{9}
0.008-1a	46	65		4.60×10^{8}	6.50×10^{9}		
0.008-1b		11		0.00	1.10×10^{9}		
0.008-2a	16	16		1.60×10^{8}	1.60×10^{9}		
0.008-2b	28	9		2.80×10^{8}	9.00×10^{8}		
0.008-3a	198	12	3	1.98×10^{9}	1.20×10^{9}	3.00×10^{9}	
0.008-3b	180	9	2	1.80×10^{9}	9.00×10^{8}	2.00×10^{9}	
							1.56×10^{9}
0.01-1a	0	10			1.00×10^{9}		
0.01-1b	42	11		4.20×10^{8}	1.10×10^{9}		
0.01-2a	74	14		7.40×10^{8}	1.40×10^{9}		
0.01-2b	62	8		6.20×10^{8}	8.00×10^{8}		
0.01-3a	1	9		1.00×10^{7}	9.00×10^{8}		
0.01-3b	0	0					
							7.77×10^{8}
Results from 22/10/03							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values
	10^{-7}	10^{-8}	10^{-9}	10^{-7}	10^{-8}	10^{-9}	
c1a	67	7	1	6.70×10^{8}	7.00×10^{8}	1.00×10^{9}	
c1b	18	9		1.80×10^{8}	9.00×10^{8}		
c2a	6	6		6.00×10^{7}	6.00×10^{8}		
c2b	30		1	3.00×10^{8}		1.00×10^{9}	
c3a	1			1.00×10^{7}			
c3b	24			2.40×10^{8}			
							5.15×10^{8}

0.002-1a		5	15		5.00×10^{-8}	1.50×10^{-10}	
0.002-1b	10			1.00×10^{-8}			
0.002-2a	362			3.62×10^{-9}			
0.002-2b	248			2.48×10^{-9}			
0.002-3a	179			1.79×10^{-9}			
0.002-3b	156			1.56×10^{-9}			
							3.58×10^{-9}
0.004-1a							
0.004-1b							
0.004-2a							
0.004-2b							
0.004-3a							
0.004-3b							
0.006-1a							
0.006-1b							
0.006-2a	39			3.90×10^{-8}			
0.006-2b	38			3.80×10^{-8}			
0.006-3a	59			5.90×10^{-8}			
0.006-3b	48			4.80×10^{-8}			
							4.60×10^{-8}
0.008-1a							
0.008-1b							
0.008-2a							
0.008-2b							

0.008-3a							
0.008-3b							
0.01-1a							
0.01-1b							
0.01-2a							
0.01-2b							
0.01-3a							
0.01-3b							
Results from 23/10/03							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values
	10^{-6}	10^{-7}	10^{-8}	10^{-6}	10^{-7}	10^{-8}	
c1a	70	10		7.00×10^{7}	1.00×10^{8}		
c1b	49	3	2	4.90×10^{7}	3.00×10^{7}	2.00×10^{8}	
c2a	148	11	2	1.48×10^{8}	1.10×10^{8}	2.00×10^{8}	
c2b	199	16	3	1.99×10^{8}	1.60×10^{8}	3.00×10^{8}	
c3a	88	6		8.80×10^{7}	6.00×10^{7}		
c3b	77	3	1	7.70×10^{7}	3.00×10^{7}	1.00×10^{8}	
							1.20×10^{8}
0.002-1a	95	6		9.50×10^{7}	6.00×10^{7}		
0.002-1b	119	6		1.19×10^{8}	6.00×10^{7}		
0.002-2a	108	8		1.08×10^{8}	8.00×10^{7}		
0.002-2b	187	5	1	1.87×10^{8}	5.00×10^{7}	1.00×10^{8}	
0.002-3a	35	2		3.50×10^{7}	2.00×10^{7}		
0.002-3b	49	5		4.90×10^{7}	5.00×10^{7}		
							7.79×10^{7}

0.004-1a	82	2		8.20×10^{-7}	2.00×10^{-7}		
0.004-1b	149	1		1.49×10^{-8}	1.00×10^{-7}		
0.004-2a	215	14		2.15×10^{-8}	1.40×10^{-8}		
0.004-2b	192	12	3	1.92×10^{-8}	1.20×10^{-8}	3.00×10^{-8}	
0.004-3a	441	30	1	4.41×10^{-8}	3.00×10^{-8}	1.00×10^{-8}	
0.004-3b	366	35		3.66×10^{-8}	3.50×10^{-8}		
							1.99×10^{-8}
0.006-1a	49	9		4.90×10^{-7}	9.00×10^{-7}		
0.006-1b	42	11		4.20×10^{-7}	1.10×10^{-8}		
0.006-2a	393	56	3	3.93×10^{-8}	5.60×10^{-8}	3.00×10^{-8}	
0.006-2b	457	67	2	4.57×10^{-8}	6.70×10^{-8}	2.00×10^{-8}	
0.006-3a	102	8	3	1.02×10^{-8}	8.00×10^{-7}	3.00×10^{-8}	
0.006-3b	94	1	3	9.40×10^{-7}	1.00×10^{-7}	3.00×10^{-8}	
							2.35×10^{-8}
0.008-1a	207	12	1	2.07×10^{-8}	1.20×10^{-8}	1.00×10^{-8}	
0.008-1b	234	16		2.34×10^{-8}	1.60×10^{-8}		
0.008-2a	829	106	4	8.29×10^{-8}	1.06×10^{-9}	4.00×10^{-8}	
0.008-2b	816	75	13	8.16×10^{-8}	7.50×10^{-8}	1.30×10^{-9}	
0.008-3a	39	1		3.90×10^{-7}	1.00×10^{-7}		
0.008-3b	118	4		1.18×10^{-8}	4.00×10^{-7}		
							4.12×10^{-8}
0.01-1a	306	1	2	3.06×10^{-8}	1.00×10^{-7}	2.00×10^{-8}	
0.01-1b	298	13	2	2.98×10^{-8}	1.30×10^{-8}	2.00×10^{-8}	
0.01-2a	65	31	3	6.50×10^{-7}	3.10×10^{-8}	3.00×10^{-8}	
0.01-2b	177	24	3	1.77×10^{-8}	2.40×10^{-8}	3.00×10^{-8}	

0.01-3a	110	48	5	1.10×10^{8}	4.80×10^{8}	5.00×10^{8}	
0.01-3b	98	40		9.80×10^{7}	4.00×10^{8}		
							2.43×10^{8}

Large portions of the data for this study were uncountable; therefore there is no graph for these data.

04/11/03 Data

Table A.5

Chitosan and MRSA data	04/11/03
initial inoculum	
cell count mean	12.2
cells per ml	4.88×10^{7}
cells in 200µl inoculum	9.76×10^{6}
Each culture for this experimental run was plated out two times (a & b)	

Results from 5/11/03							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values
	10^{-7}	10^{-8}	10^{-9}	10^{-7}	10^{-8}	10^{-9}	
c1a	10	11	1	1.00×10^{8}	1.10×10^{9}	1.00×10^{9}	
c1b	50		35	5.00×10^{8}		3.50×10^{10}	
c2a	24	4	2	2.40×10^{8}	4.00×10^{8}	2.00×10^{9}	
c2b	26	6	1	2.60×10^{8}	6.00×10^{8}	1.00×10^{9}	
c3a	68	13		6.80×10^{8}	1.30×10^{9}		
c3b	103	11		1.03×10^{9}	1.10×10^{9}		
							3.09×10^{9}
0.02 - 1a	80	2		8.00×10^{8}	2.00×10^{8}		
0.02 - 1b	54			5.40×10^{8}			
0.02 - 2a	64	8	3	6.40×10^{8}	8.00×10^{8}	3.00×10^{9}	
0.02 - 2b	87	10	1	8.70×10^{8}	1.00×10^{9}	1.00×10^{9}	
0.02 - 3a	60	7		6.00×10^{8}	7.00×10^{8}		

0.02 - 3b	66	8		6.60×10^{8}	8.00×10^{8}		
							8.93×10^{8}
0.04 - 1a	27	5		2.70×10^{8}	5.00×10^{8}		
0.04 - 1b	40	6		4.00×10^{8}	6.00×10^{8}		
0.04 - 2a	257	33	1	2.57×10^{9}	3.30×10^{9}	1.00×10^{9}	
0.04 - 2b	181	32	2	1.81×10^{9}	3.20×10^{9}	2.00×10^{9}	
0.04 - 3a	149	17		1.49×10^{9}	1.70×10^{9}		
0.04 - 3b	168	24	3	1.68×10^{9}	2.40×10^{9}	3.00×10^{9}	
							1.73×10^{9}
0.06 - 1a	62	2		6.20×10^{8}	2.00×10^{8}		
0.06 - 1b	41	8		4.10×10^{8}	8.00×10^{8}		
0.06 - 2a	4	2		4.00×10^{7}	2.00×10^{8}		
0.06 - 2b	24		2	2.40×10^{8}		2.00×10^{9}	
0.06 - 3a	79	3	2	7.90×10^{8}	3.00×10^{8}	2.00×10^{9}	
0.06 - 3b	93	8	2	9.30×10^{8}	8.00×10^{8}	2.00×10^{9}	
							8.09×10^{8}
0.08 - 1a	196	21	2	1.96×10^{9}	2.10×10^{9}	2.00×10^{9}	
0.08 - 1b	212	26		2.12×10^{9}	2.60×10^{9}		
0.08 - 2a	59	5		5.90×10^{8}	5.00×10^{8}		
0.08 - 2b	78	4	3	7.80×10^{8}	4.00×10^{8}	3.00×10^{9}	
0.08 - 3a	346	40	4	3.46×10^{9}	4.00×10^{9}	4.00×10^{9}	
0.08 - 3b	350	33		3.50×10^{9}	3.30×10^{9}		
							2.29×10^{9}
0.1 - 1a	83	2		8.30×10^{8}	2.00×10^{8}		

0.1 - 1b	7	2		7.00×10^{7}	2.00×10^{8}		
0.1 - 2a	14	3		1.40×10^{8}	3.00×10^{8}		
0.1 - 2b	45	7	1	4.50×10^{8}	7.00×10^{8}	1.00×10^{9}	
0.1 - 3a	90	4		9.00×10^{8}	4.00×10^{8}		
0.1 - 3b	95	8	1	9.50×10^{8}	8.00×10^{8}	1.00×10^{9}	
							5.67×10^{8}
Results from 6/11/03							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values
	10^{-6}	10^{-7}	10^{-8}	10^{-6}	10^{-7}	10^{-8}	
c1a		25	4	0.00	2.50×10^{8}	4.00×10^{8}	
c1b	837	40	5	8.37×10^{8}	4.00×10^{8}	5.00×10^{8}	
c2a	693		3	6.93×10^{8}		3.00×10^{8}	
c2b	580	76	8	5.80×10^{8}	7.60×10^{8}	8.00×10^{8}	
c3a		79	4		7.90×10^{8}	4.00×10^{8}	
c3b		73	15		7.30×10^{8}	1.50×10^{9}	
							6.91×10^{8}
0.02 - 1a	501	51	4	5.01×10^{8}	5.10×10^{8}	4.00×10^{8}	
0.02 - 1b	405	52	2	4.05×10^{8}	5.20×10^{8}	2.00×10^{8}	
0.02 - 2a		280	36		2.80×10^{9}	3.60×10^{9}	
0.02 - 2b		246	27		2.46×10^{9}	2.70×10^{9}	
0.02 - 3a		66	14		6.60×10^{8}	1.40×10^{9}	
0.02 - 3b	651	60		6.51×10^{8}	6.00×10^{8}		
							1.24×10^{9}
0.04 - 1a	59	5		5.90×10^{7}	5.00×10^{7}		
0.04 - 1b		4			4.00×10^{7}		
0.04 - 2a		159	11		1.59×10^{9}	1.10×10^{9}	
0.04 - 2b		149	9		1.49×10^{9}	9.00×10^{8}	

0.04 - 3a	3408	328	24	3.41×10^{9}	3.28×10^{9}	2.40×10^{9}	
0.04 - 3b	2984	321	26	2.98×10^{9}	3.21×10^{9}	2.60×10^{9}	
							1.78×10^{9}
0.06 - 1a	208	32	2	2.08×10^{8}	3.20×10^{8}	2.00×10^{8}	
0.06 - 1b	186	60	6	1.86×10^{8}	6.00×10^{8}	6.00×10^{8}	
0.06 - 2a	724	96	8	7.24×10^{8}	9.60×10^{8}	8.00×10^{8}	
0.06 - 2b		43	14		4.30×10^{8}	1.40×10^{9}	
0.06 - 3a		104	10		1.04×10^{9}	1.00×10^{9}	
0.06 - 3b		117	12		1.17×10^{9}	1.20×10^{9}	
							7.23×10^{8}
0.08 - 1a		330	38		3.30×10^{9}	3.80×10^{9}	
0.08 - 1b		371	44		3.71×10^{9}	4.40×10^{9}	
0.08 - 2a		95	10		9.50×10^{8}	1.00×10^{9}	
0.08 - 2b		118	12		1.18×10^{9}	1.20×10^{9}	
0.08 - 3a		403	42		4.03×10^{9}	4.20×10^{9}	
0.08 - 3b		399	39		3.99×10^{9}	3.90×10^{9}	
							2.97×10^{9}
0.1 - 1a	506	23	8	5.06×10^{8}	2.30×10^{8}	8.00×10^{8}	
0.1 - 1b		58	9		5.80×10^{8}	9.00×10^{8}	
0.1 - 2a	183	17	4	1.83×10^{8}	1.70×10^{8}	4.00×10^{8}	
0.1 - 2b	120	16	3	1.20×10^{8}	1.60×10^{8}	3.00×10^{8}	
0.1 - 3a	92	11	2	9.20×10^{7}	1.10×10^{8}	2.00×10^{8}	
0.1 - 3b	125	15	3	1.25×10^{8}	1.50×10^{8}	3.00×10^{8}	
							3.13×10^{8}
Results from 7/11/03							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values

	10^{-6}	10^{-7}	10^{-8}	10^{-6}	10^{-7}	10^{-8}	
c1a	79	21		7.90×10^{-7}	2.10×10^{8}		
c1b		23			2.30×10^{8}		
c2a		89			8.90×10^{8}		
c2b		91			9.10×10^{8}		
c3a		183			1.83×10^{9}		
c3b		185			1.85×10^{9}		
							1.14×10^{9}
0.02 - 1a	256	34		2.56×10^{8}	3.40×10^{8}		
0.02 - 1b	284	36		2.84×10^{8}	3.60×10^{8}		
0.02 - 2a		297			2.97×10^{9}		
0.02 - 2b		291			2.91×10^{9}		
0.02 - 3a	538	40		5.38×10^{8}	4.00×10^{8}		
0.02 - 3b	536	58		5.36×10^{8}	5.80×10^{8}		
							9.17×10^{8}
0.04 - 1a	508	47		5.08×10^{8}	4.70×10^{8}		
0.04 - 1b	535	56		5.35×10^{8}	5.60×10^{8}		
0.04 - 2a	222	16		2.22×10^{8}	1.60×10^{8}		
0.04 - 2b	36	11		3.60×10^{7}	1.10×10^{8}		
0.04 - 3a	693	100		6.93×10^{8}	1.00×10^{9}		
0.04 - 3b	778	106		7.78×10^{8}	1.06×10^{9}		
							5.11×10^{8}
0.06 - 1a	565			5.65×10^{8}			
0.06 - 1b	363			3.63×10^{8}			
0.06 - 2a	314	25		3.14×10^{8}	2.50×10^{8}		
0.06 - 2b	297	15		2.97×10^{8}	1.50×10^{8}		

0.06 - 3a		30		0.00	3.00×10^8		
0.06 - 3b		41		0.00	4.10×10^8		
							2.65×10^8
0.08 - 1a	477	23		4.77×10^8	2.30×10^8		
0.08 - 1b	350	19		3.50×10^8	1.90×10^8		
0.08 - 2a		190			1.90×10^9		
0.08 - 2b		101			1.01×10^9		
0.08 - 3a	992	79		9.92×10^8	7.90×10^8		
0.08 - 3b	673	62		6.73×10^8	6.20×10^8		
							7.23×10^8
0.1 - 1a	588	71		5.88×10^8	7.10×10^8		
0.1 - 1b	708	90		7.08×10^8	9.00×10^8		
0.1 - 2a		50			5.00×10^8		
0.1 - 2b	657	47		6.57×10^8	4.70×10^8		
0.1 - 3a	543	60		5.43×10^8	6.00×10^8		
0.1 - 3b	394	50		3.94×10^8	5.00×10^8		
							5.97×10^8

18/01/05 Data

Chitosan and MRSA data				18/01/05			
initial inoculum							
cell count mean				23.5			
cells per ml				9.40×10^7			
cells in 200µl inoculum				1.88×10^7			
Results from the 19th Jan 05							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values
	10^{-6}	10^{-7}	10^{-8}	10^{-6}	10^{-7}	10^{-8}	
c1		262	16		2.62×10^9	1.60×10^9	

c2			105			1.05×10^{-10}	
c3		572	62		5.72×10^{-9}	6.20×10^{-9}	
							5.33×10^{-9}
0.02 - 1		365	59		3.65×10^{-9}	5.90×10^{-9}	
0.02 - 2			139			1.39×10^{-10}	
0.02 - 3			36			3.60×10^{-9}	
							6.76×10^{-9}
0.04 - 1			179			1.79×10^{-10}	
0.04 - 2		904			9.04×10^{-9}		
0.04 - 3			166			1.66×10^{-10}	
							1.45×10^{-10}
0.06 - 1		423	47		4.23×10^{-9}	4.70×10^{-9}	
0.06 - 2			145			1.45×10^{-10}	
0.06 - 3			155			1.55×10^{-10}	
							9.73×10^{-9}
0.08 - 1	5560			5.56×10^{-9}			
0.08 - 2		268	36		2.68×10^{-9}	3.60×10^{-9}	
0.08 - 3	2984			2.98×10^{-9}			
							3.71×10^{-9}
0.1 - 1			151			1.51×10^{-10}	
0.1 - 2			173			1.73×10^{-10}	
0.1 - 3		376	118		3.76×10^{-9}	1.18×10^{-10}	
							1.20×10^{-10}
Results from the 20th							
	cell counts from plate			cells per ml for these values (by			Average of cells

	count agars (by dilution)			dilution)			per ml values
	10^{-7}	10^{-8}	10^{-9}	10^{-7}	10^{-8}	10^{-9}	
c1	117	15	1	1.17×10^{9}	1.50×10^{9}	1.00×10^{9}	
c2		68	10		6.80×10^{9}	1.00×10^{10}	
c3	120	16	1	1.20×10^{9}	1.60×10^{9}	1.00×10^{9}	
							3.03×10^{9}
0.02 - 1			34			3.40×10^{10}	
0.02 - 2		92	2		9.20×10^{9}	2.00×10^{9}	
0.02 - 3		40	6		4.00×10^{9}	6.00×10^{9}	
							1.10×10^{10}
0.04 - 1		53	16		5.30×10^{9}	1.60×10^{10}	
0.04 - 2		300	13		3.00×10^{10}	1.30×10^{10}	
0.04 - 3		75	15		7.50×10^{9}	1.50×10^{10}	
							1.45×10^{10}
0.06 - 1	447	54	8	4.47×10^{9}	5.40×10^{9}	8.00×10^{9}	
0.06 - 2							
0.06 - 3	507			5.07×10^{9}			
							5.74×10^{9}
0.08 - 1		48	8		4.80×10^{9}	8.00×10^{9}	
0.08 - 2		32			3.20×10^{9}		
0.08 - 3		78	9		7.80×10^{9}	9.00×10^{9}	
							6.56×10^{9}
0.1 - 1	471	57	6	4.71×10^{9}	5.70×10^{9}	6.00×10^{9}	
0.1 - 2	547	90	5	5.47×10^{9}	9.00×10^{9}	5.00×10^{9}	

0.1 - 3		69	5		6.90×10^{9}	5.00×10^{9}	
							5.97×10^{9}
Results from the 23rd							
	cell counts from plate count agars (by dilution)			cells per ml for these values (by dilution)			Average of cells per ml values
	10^{-6}	10^{-7}	10^{-8}	10^{-6}	10^{-7}	10^{-8}	
c1		17			1.70×10^{8}		
c2		89	11		8.90×10^{8}	1.10×10^{9}	
c3		22	3		2.20×10^{8}	3.00×10^{8}	
							5.36×10^{8}
0.02 - 1			60			6.00×10^{9}	
0.02 - 2		285	34		2.85×10^{9}	3.40×10^{9}	
0.02 - 3							
							4.08×10^{9}
0.04 - 1			24			2.40×10^{9}	
0.04 - 2			387			3.87×10^{10}	
0.04 - 3			66			6.60×10^{9}	
							1.59×10^{10}
0.06 - 1			56			5.60×10^{9}	
0.06 - 2		111	6		1.11×10^{9}	6.00×10^{8}	
0.06 - 3			35			3.50×10^{9}	
							2.70×10^{9}
0.08 - 1			66			6.60×10^{9}	
0.08 - 2			51			5.10×10^{9}	
0.08 - 3			36			3.60×10^{9}	
							5.10×10^{9}

0.1 - 1		80	4		8.00×10^{-8}	4.00×10^{-8}	
0.1 - 2		39	4		3.90×10^{-8}	4.00×10^{-8}	
0.1 - 3		135	12		1.35×10^{-9}	1.20×10^{-9}	
							7.57×10^{-8}

B. PLA pore size data

PLA pore size measurement data

Table B.1 pore size measurement from fig 4.24 (PLA 1)

Area of picture			
Width of printed out PLA image (cm)	Real width of PLA image measured (µm)	Height of printed out PLA image (cm)	Real height of PLA image measured (µm)
20.3	31.72	20.36	31.81
Real area (µm^2)=	1009.05		
pore size measurements			
Diameter of pore on printout (cm)	Real pore Diameter (µm)	Area of pore (pie x (Diameter)) (µm^2)	
0.9	1.41	4.42	
1.2	1.88	5.89	
1.5	2.34	7.36	
1.1	1.72	5.40	
0.9	1.41	4.42	
0.8	1.25	3.93	
1	1.56	4.91	
3.8	5.94	18.65	
1.3	2.03	6.38	
2	3.13	9.82	
0.7	1.09	3.44	
0.8	1.25	3.93	
1.4	2.19	6.87	
0.7	1.09	3.44	
4.3	6.72	21.11	
2.7	4.22	13.25	
2.5	3.91	12.27	
1	1.56	4.91	
2.7	4.22	13.25	
1.5	2.34	7.36	
0.7	1.09	3.44	
0.6	0.94	2.95	
0.6	0.94	2.95	
3.3	5.16	16.20	
1.5	2.34	7.36	
0.7	1.09	3.44	
0.6	0.94	2.95	
1	1.56	4.91	
0.8	1.25	3.93	
0.8	1.25	3.93	

2.1	3.28	10.31	
2	3.13	9.82	
1.5	2.34	7.36	
2.1	3.28	10.31	
0.6	0.94	2.95	
2	3.13	9.82	
1.8	2.81	8.84	
1	1.56	4.91	
1.7	2.66	8.34	
2	3.13	9.82	
0.7	1.09	3.44	
0.6	0.94	2.95	
2	3.13	9.82	
1.3	2.03	6.38	
1.8	2.81	8.84	
3.2	5.00	15.71	
2.5	3.91	12.27	
1.3	2.03	6.38	
1	1.56	4.91	
1.8	2.81	8.84	
1.1	1.72	5.40	
1.3	2.03	6.38	
0.7	1.09	3.44	
2	3.13	9.82	
2.3	3.59	11.29	
0.7	1.09	3.44	
0.6	0.94	2.95	
1.7	2.66	8.34	
0.5	0.78	2.45	
0.4	0.63	1.96	
0.9	1.41	4.42	
1.4	2.19	6.87	
0.9	1.41	4.42	
1.3	2.03	6.38	
0.7	1.09	3.44	
2	3.13	9.82	
1.9	2.97	9.33	
1	1.56	4.91	
1.5	2.34	7.36	
0.7	1.09	3.44	
0.9	1.41	4.42	
1.3	2.03	6.38	
0.9	1.41	4.42	
2.4	3.75	11.78	
2	3.13	9.82	
0.7	1.09	3.44	
2.4	3.75	11.78	
1	1.56	4.91	

1.7	2.66	8.34	
1.4	2.19	6.87	
	Average Pore area (μm^2)	7.04	
	Total area of pores (μm^2)	563.03	
	Percentage pores (%)	55.80	
	St Dev of pore area	3.88	

Table B.2 pore size measurement from fig 4.25 (PLA 2)

Area of picture			
Width of printed out PLA image (cm)	Real width of PLA image measured (µm)	Height of printed out PLA image (cm)	Real height of PLA image measured (µm)
20.3	31.72	20.36	31.81
Real area (µm^2)=	1009.05		
pore size measurements			
Diameter of pore on printout (cm)	Real pore Diameter (µm)	Area of pore (pie x (Diameter)) (µm^2)	
2.7	4.22	13.25	
2.8	4.38	13.74	
1.8	2.81	8.84	
2.2	3.44	10.80	
2	3.13	9.82	
0.9	1.41	4.42	
0.8	1.25	3.93	
2.5	3.91	12.27	
1.9	2.97	9.33	
1.8	2.81	8.84	
1.2	1.88	5.89	
1	1.56	4.91	
0.7	1.09	3.44	
0.9	1.41	4.42	
1.1	1.72	5.40	
2.9	4.53	14.24	
0.6	0.94	2.95	
0.5	0.78	2.45	
1.2	1.88	5.89	
3.6	5.63	17.67	
0.8	1.25	3.93	
3.3	5.16	16.20	
0.9	1.41	4.42	
1	1.56	4.91	
1.2	1.88	5.89	
0.5	0.78	2.45	
0.5	0.78	2.45	
0.6	0.94	2.95	
0.4	0.63	1.96	
2	3.13	9.82	
4.1	6.41	20.13	
1.7	2.66	8.34	
1	1.56	4.91	
0.6	0.94	2.95	

0.5	0.78	2.45
0.7	1.09	3.44
1	1.56	4.91
1.1	1.72	5.40
1	1.56	4.91
0.8	1.25	3.93
4.3	6.72	21.11
1.3	2.03	6.38
0.7	1.09	3.44
0.9	1.41	4.42
1	1.56	4.91
0.6	0.94	2.95
0.9	1.41	4.42
0.7	1.09	3.44
0.5	0.78	2.45
2.1	3.28	10.31
5.5	8.59	27.00
1.5	2.34	7.36
1.5	2.34	7.36
3.6	5.63	17.67

Average Pore area(μm^2)	7.44
Total area of pores (μm^2)	402.03
Percentage pores (%)	39.84
St Dev of pore area	5.56

TableB.3 pore size measurement from fig 4.26 (PLA 3)

Area of picture			
Width of printed out PLA image (cm)	Real width of PLA image measured (µm)	Height of printed out PLA image (cm)	Real height of PLA image measured (µm)
20.3	31.72	20.36	31.81
Real area (µm^2)=	1009.05		
pore size measurements			
Diameter of pore on printout (cm)	Real pore Diameter (µm)	Area of pore (pie x (Diameter)) (µm^2)	
2.5	3.91	12.27	
1.7	2.66	8.34	
1.3	2.03	6.38	
1.3	2.03	6.38	
1.3	2.03	6.38	
1.8	2.81	8.84	
1.9	2.97	9.33	
0.9	1.41	4.42	
2	3.13	9.82	
4.7	7.34	23.07	
1.2	1.88	5.89	
1.8	2.81	8.84	
1.3	2.03	6.38	
6.1	9.53	29.94	
2	3.13	9.82	
0.6	0.94	2.95	
5.5	8.59	27.00	
3.2	5.00	15.71	
5	7.81	24.54	
7.2	11.25	35.34	
1.2	1.88	5.89	
1.5	2.34	7.36	
2.5	3.91	12.27	
1.1	1.72	5.40	
1.2	1.88	5.89	
5.9	9.22	28.96	
1.2	1.88	5.89	
0.6	0.94	2.95	
1.2	1.88	5.89	
3	4.69	14.73	
2.1	3.28	10.31	
1.4	2.19	6.87	
2.5	3.91	12.27	
1.1	1.72	5.40	
2.5	3.91	12.27	

1.8	2.81	8.84
5.8	9.06	28.47
4.7	7.34	23.07

Average Pore area(μm^2)	12.22
Total area of pores (μm^2)	464.37
Percentage pores (%)	46.02
St Dev of pore area	8.71

C. Tissue culture study

C.1 Tissue culture study raw data

Assumptions

All of the samples measure 30mm in length. Each sample was tested 9 times. All plasma treatment work was performed on the Riccarton apparatus unless stated otherwise.

Key

Grey table cells, couldn't be seen clearly enough to measure. Media Red = no change in the pH of the media. This means that the media is not being metabolised much/ at all by viable cells. Media Orange = slight change in the pH of the media. This means that the media is being metabolised by cells in the flask. 21/08/2005, no data for inoculum. An oversight due to late working hours and heavy work load.

Polypropylene Data

	Inoculation /cell count day (cells per ml)	Cell growth up biomaterial strip (mm)			
Day	0	8	15	22	29
Date	20/06/2006	28/06/2006	05/07/2006	12/07/2006	19/07/2006
Polypropylene 1	140000	0.00	0.00	0.00	0.00
Polypropylene 2	140000	0.00	0.00	0.00	0.00
Polypropylene 3	140000	0.00	0.00	0.00	0.00
Polypropylene treated with Argon plasma 1	140000	0.00	0.00	0.00	0.00
Polypropylene treated with Argon plasma 2	140000	0.00	0.00	0.00	0.00
Polypropylene treated with Argon plasma 3	140000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma 1	140000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma 2	140000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma 3	140000	0.00	0.00	0.00	0.00
Polypropylene coated with 0.1% Chitosan solution 1	140000	0.00	0.00	0.00	0.00
Polypropylene coated with 0.1% Chitosan solution 2	140000	1.00	1.20	2.80	2.80
Polypropylene coated with 0.1% Chitosan solution 3	140000	3.50	2.50	5.00	5.00
Polypropylene treated with Argon plasma then coated with 0.1% Chitosan solution 1	140000	0.00	0.20	0.00	0.00
Polypropylene treated with	140000	0.00	0.00	0.00	0.00

Argon plasma then coated with 0.1% Chitosan solution 2					
Polypropylene treated with Argon plasma then coated with 0.1% Chitosan solution 3	140000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma then coated with 0.1% Chitosan solution 1	140000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma then coated with 0.1% Chitosan solution 2	140000				
Polypropylene treated with Ammonia plasma then coated with 0.1% Chitosan solution 3	140000	0.00	0.00	0.00	0.00

Day	0	8	15	22	29
Date	18/07/2006	26/07/2006	02/08/2006	09/08/2006	16/08/2006
Polypropylene 1	330000	0.00	0.00	0.00	0.00
Polypropylene 2	330000	0.00	0.00	0.00	0.00
Polypropylene 3	330000	0.00	0.00	0.00	0.00
Polypropylene treated with Argon plasma 1	330000	0.00	16.00	19.00	24.00
Polypropylene treated with Argon plasma 2	330000	0.75	7.00	15.50	24.00
Polypropylene treated with Argon plasma 3	330000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma 1	330000	1.50	10.00	16.00	28.00
Polypropylene treated with Ammonia plasma 2	330000	5.00	8.00	15.00	26.00
Polypropylene treated with Ammonia plasma 3	330000	1.00	24.00	15.00	20.00
Polypropylene coated with 0.1% Chitosan solution 1	330000	0.00	0.00	0.00	0.00

Polypropylene coated with 0.1% Chitosan solution 2	330000	4.50	11.50	19.00	19.00
Polypropylene coated with 0.1% Chitosan solution 3	330000	0.50	1.00	0.00	0.00
Polypropylene treated with Argon plasma then coated with 0.1% Chitosan solution 1	330000	0.00	0.00	0.00	0.00
Polypropylene treated with Argon plasma then coated with 0.1% Chitosan solution 2	330000	2.00	0.00	0.00	0.00
Polypropylene treated with Argon plasma then coated with 0.1% Chitosan solution 3	330000	0.50	4.00	2.00	2.00
Polypropylene treated with Ammonia plasma then coated with 0.1% Chitosan solution 1	330000	1.00	9.00	16.00	30.00
Polypropylene treated with Ammonia plasma then coated with 0.1% Chitosan solution 2	330000	0.00	10.00	15.50	15.50
Polypropylene treated with Ammonia plasma then coated with 0.1% Chitosan solution 3	330000	0.00	0.00	0.00	0.00

Day	0	8	15	22	29
Date	25/08/2006	02/09/2006	09/09/2006	16/09/2006	23/09/2006
Polypropylene 1	190000	0.00	0.00	0.00	0.00
Polypropylene 2	190000	0.00	0.00	30.00	30.00
Polypropylene 3	190000	0.00	3.13	30.00	30.00
Polypropylene treated with Argon plasma 1	190000	0.00	0.00	0.00	0.00
Polypropylene treated with Argon plasma 2	190000	0.00	2.00	30.00	30.00
Polypropylene treated with Argon plasma 3	190000	0.00	0.00	12.00	30.00

Polypropylene treated with Ammonia plasma 1	190000	3.00	0.50	20.00	30.00
Polypropylene treated with Ammonia plasma 2	190000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma 3	190000	1.50	4.00	5.00	30.00
Polypropylene coated with 0.1% Chitosan solution 1	190000	0.00	4.00	30.00	30.00
Polypropylene coated with 0.1% Chitosan solution 2	190000	1.00	3.75	22.50	30.00
Polypropylene coated with 0.1% Chitosan solution 3	190000	0.00	0.00	0.00	0.00
Polypropylene treated with Argon plasma then coated with 0.1% Chitosan solution 1	190000	9.00	14.00	30.00	30.00
Polypropylene treated with Argon plasma then coated with 0.1% Chitosan solution 2	190000	0.00	0.00	0.00	0.00
Polypropylene treated with Argon plasma then coated with 0.1% Chitosan solution 3	190000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma then coated with 0.1% Chitosan solution 1	190000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma then coated with 0.1% Chitosan solution 2	190000	0.00	0.00	0.00	0.00
Polypropylene treated with Ammonia plasma then coated with 0.1% Chitosan solution 3	190000	0.00	4.00	4.00	4.00

Polyurethane Data

Day	0	8	15	22	29
Date	14/07/2005	22/07/2005	29/07/2005	05/08/2005	12/08/2005
Tuftane® Polyurethane 1	170000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane 2	170000	0.00	0.00	4.00	14.00
Tuftane® Polyurethane 3	170000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Argon plasma 1	170000	5.20	24.00	30.00	30.00
Tuftane® Polyurethane treated with Argon plasma 2	170000	4.00	30.00	30.00	30.00
Tuftane® Polyurethane treated with Argon plasma 3	170000	0.00	1.00	6.00	10.00
Tuftane® Polyurethane treated with Ammonia plasma 1	170000	0.00	0.00	30.00	26.00
Tuftane® Polyurethane treated with Ammonia plasma 2	170000	5.00	9.00	11.00	16.00
Tuftane® Polyurethane treated with Ammonia plasma 3	170000	0.50	6.25	28.00	30.00
Tuftane® Polyurethane dusted with Chitosan powder then treated with Argon plasma 1	170000	2.50	6.00	30.00	30.00
Tuftane® Polyurethane dusted with Chitosan powder then treated with Argon plasma 2	170000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane dusted with Chitosan powder then treated with Argon plasma 3	170000	0.00	0.00	0.00	2.25

Day	0	8	15	22	29
Date	21/08/2005	29/08/2005	05/09/2005	12/09/2005	19/09/2005
Tuftane® Polyurethane 1		0.00	0.00	0.00	0.00
Tuftane® Polyurethane 2		2.00	4.50	14.00	30.00

Tuftane® Polyurethane 3		0.00	0.20	4.50	24.00
Tuftane® Polyurethane treated with Argon plasma 1		7.75	30.00	30.00	30.00
Tuftane® Polyurethane treated with Argon plasma 2		0.00	0.40	0.00	0.00
Tuftane® Polyurethane treated with Argon plasma 3		6.50	30.00	30.00	30.00
Tuftane® Polyurethane treated with Ammonia plasma 1		0.00	30.00	30.00	30.00
Tuftane® Polyurethane treated with Ammonia plasma 2		0.00	2.00	15.00	30.00
Tuftane® Polyurethane treated with Ammonia plasma 3		0.00	6.00	30.00	30.00
Tuftane® Polyurethane dusted with Chitosan powder then treated with Argon plasma 1		0.00	30.00	30.00	30.00
Tuftane® Polyurethane dusted with Chitosan powder then treated with Argon plasma 2		2.00	0.00	1.00	0.00
Tuftane® Polyurethane dusted with Chitosan powder then treated with Argon plasma 3		0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Argon plasma on the Europlasma machine 1		14.00	30.00	30.00	30.00
Tuftane® Polyurethane treated with Argon plasma on the Europlasma machine 2		0.00	6.00	0.00	6.00
Tuftane® Polyurethane treated with Argon plasma on the Europlasma machine 3		0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Argon plasma on the Europlasma machine 4		0.00	0.00	0.00	0.00

Tuftane® Polyurethane treated with Argon plasma on the Europlasma machine 5		7.00	30.00	30.00	30.00
Tuftane® Polyurethane treated with Argon plasma on the Europlasma machine 6		0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Oxygen plasma on the Europlasma machine 1		0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Oxygen plasma on the Europlasma machine 2		0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Oxygen plasma on the Europlasma machine 3		0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Oxygen plasma on the Europlasma machine 4		0.00	9.00	30.00	30.00
Tuftane® Polyurethane treated with Oxygen plasma on the Europlasma machine 5		4.00	8.50	23.00	30.00
Tuftane® Polyurethane treated with Oxygen plasma on the Europlasma machine 6		0.00	0.00	0.00	0.00

Day	0	8	15	22	29
Date	25/08/2005	02/09/2005	09/09/2005	16/09/2005	23/09/2005
Tuftane® Polyurethane 1	240000	4.00	4.00	21.00	25.00
Tuftane® Polyurethane 2	240000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane 3	240000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Argon plasma 1	240000	0.00	0.00	0.00	2.00
Tuftane® Polyurethane treated with Argon plasma 2	240000	0.00	0.00	0.00	0.00

Tuftane® Polyurethane treated with Argon plasma 3	240000	5.00	18.00	30.00	30.00
Tuftane® Polyurethane treated with Ammonia plasma 1	240000	1.00	2.50	6.00	5.25
Tuftane® Polyurethane treated with Ammonia plasma 2	240000	5.50	16.00	25.75	30.00
Tuftane® Polyurethane treated with Ammonia plasma 3	240000	3.00	8.00	24.00	30.00
Tuftane® Polyurethane dusted with Chitosan powder then treated with Argon plasma 1	240000	0.00	8.00	13.50	13.50
Tuftane® Polyurethane dusted with Chitosan powder then treated with Argon plasma 2	240000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane dusted with Chitosan powder then treated with Argon plasma 3	240000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Argon plasma on the Europlasma machine 1	240000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Argon plasma on the Europlasma machine 2	240000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Argon plasma on the Europlasma machine 3	240000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Oxygen plasma on the Europlasma machine 1	240000	8.00	3.00	16.00	12.00
Tuftane® Polyurethane treated with Oxygen plasma on the Europlasma machine 2	240000	0.00	0.00	0.00	0.00
Tuftane® Polyurethane treated with Oxygen plasma on the Europlasma machine 3	240000	4.00	16.00	28.00	30.00

Polyester Data

Day	0	8	15	22	29
Date	16/07/2005	24/07/2005	31/07/2005	07/08/2005	14/08/2005
Vascutek Polyester 1	100000	3.00	3.00	4.20	6.00
Vascutek Polyester 2	100000	3.00	4.25	12.00	30.00
Vascutek Polyester 3	100000	4.40	5.50	8.00	30.00
Vascutek Polyester treated with Argon plasma 1	100000	0.00	0.00	0.00	0.00
Vascutek Polyester treated with Argon plasma 2	100000	4.00	5.00	6.00	6.00
Vascutek Polyester treated with Argon plasma 3	100000	0.00	0.00	0.00	0.00
Vascutek Polyester treated with Ammonia plasma 1	100000	0.00	0.00	0.00	0.00
Vascutek Polyester treated with Ammonia plasma 2	100000	0.00	0.00	0.00	0.00
Vascutek Polyester treated with Ammonia plasma 3	100000	0.00	0.00	0.00	0.00

Day	0	8	15	22	29
Date	03/08/2005	11/08/2005	18/08/2005	25/08/2005	01/09/2005
Vascutek Polyester 1	235000	2.50	4.00	5.00	7.50
Vascutek Polyester 2	235000	0.00	0.00	0.00	0.00
Vascutek Polyester 3	235000	2.20	3.00	4.00	6.88
Vascutek Polyester treated with Argon plasma 1	235000	0.00	0.00	2.50	4.00
Vascutek Polyester treated with Argon plasma 2	235000	0.00	0.00	0.00	0.00
Vascutek Polyester treated with Argon plasma 3	235000	0.00	0.00	0.00	0.00
Vascutek Polyester treated with Ammonia plasma 1	235000	0.00	0.00	0.00	0.00

Vascutek Polyester treated with Ammonia plasma 2	235000	0.00	0.00	0.00	0.00
Vascutek Polyester treated with Ammonia plasma 3	235000	0.00	0.00	3.00	7.20

Day	0	8	15	22	29
Date	21/08/2005	29/08/2005	05/09/2005	12/09/2005	19/09/2005
Vascutek Polyester 1		2.75	3.50	8.00	30.00
Vascutek Polyester 2		4.00	4.00	9.00	26.00
Vascutek Polyester 3		3.00	4.00	5.00	7.00
Vascutek Polyester treated with Argon plasma 1		0.00	0.00	0.00	0.00
Vascutek Polyester treated with Argon plasma 2		0.00	0.00	0.00	0.00
Vascutek Polyester treated with Argon plasma 3		3.00	5.50	6.50	8.00
Vascutek Polyester treated with Ammonia plasma 1		4.00	7.00	6.00	9.00
Vascutek Polyester treated with Ammonia plasma 2		0.00	0.00	5.75	7.00
Vascutek Polyester treated with Ammonia plasma 3		4.00	5.20	30.00	30.00

Poly-ε-caprolactone Data

Day	0	8	15	22	29
Date	14/07/2005	20/07/2005	29/07/2005	05/08/2005	12/08/2005
Poly-ε-caprolactone 6400 1	170000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 2	170000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 3	170000	0.00	0.00	0.00	0.40

Poly-ε-caprolactone 6400 treated with Argon plasma 1	170000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Argon plasma 2	170000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Argon plasma 3	170000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Ammonia plasma 1	170000	0.00	2.00	4.00	6.00
Poly-ε-caprolactone 6400 treated with Ammonia plasma 2	170000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Ammonia plasma 3	170000	0.00	0.00	0.00	0.00

Day	0	8	15	22	29
Date	18/07/2005	26/07/2005	02/08/2005	09/08/2005	16/08/2005
Poly-ε-caprolactone 6400 1	330000	1.00	2.00	0.00	0.00
Poly-ε-caprolactone 6400 2	330000	2.00	8.00	9.00	6.00
Poly-ε-caprolactone 6400 3	330000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Argon plasma 1	330000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Argon plasma 2	330000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Argon plasma 3	330000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Ammonia plasma 1	330000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Ammonia plasma 2	330000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Ammonia plasma 3	330000	0.00	0.00	0.00	0.00

Day	0	8	15	22	29
Date	25/08/2005	02/09/2005	09/09/2005	16/09/2005	23/09/2005
Poly-ε-caprolactone 6400 1	240000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 2	240000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 3	240000	0.00	0.00	0.00	0.00
Poly-ε-caprolactone 6400 treated with Argon plasma 1	240000	0.00	8.00	8.00	8.00
Poly-ε-caprolactone 6400 treated with Argon plasma 2	240000				
Poly-ε-caprolactone 6400 treated with Argon plasma 3	240000				
Poly-ε-caprolactone 6400 treated with Ammonia plasma 1	240000	0.00	5.00	10.00	30.00
Poly-ε-caprolactone 6400 treated with Ammonia plasma 2	240000				
Poly-ε-caprolactone 6400 treated with Ammonia plasma 3	240000	0.00	5.00	10.00	30.00

Solanyl Flexibilitis Data

Day	0	8	15	22	29
Date	20/06/2005	28/06/2005	05/07/2005	12/07/2005	19/07/2005
Solanyl Flexibilitis 1	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis 2	140000	1.50	0.00	0.00	0.00
Solanyl Flexibilitis 3	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Argon plasma 1	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Argon plasma 2	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Argon plasma 3	140000	0.00	0.00	0.00	0.00

Solanyl Flexibilitis treated with Ammonia plasma 1	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Ammonia plasma 2	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Ammonia plasma 3	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis extruded with 2% Chitosan powder (w/w) 1	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis extruded with 2% Chitosan powder (w/w) 2	140000	0.50	0.00	0.00	0.00
Solanyl Flexibilitis extruded with 2% Chitosan powder (w/w) 3	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis coated with 0.1% Chitosan solution 1	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis coated with 0.1% Chitosan solution 2	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis coated with 0.1% Chitosan solution 3	140000	0.00	0.00	0.00	2.00
Solanyl Flexibilitis treated with Argon plasma then coated with 0.1% Chitosan solution 1	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Argon plasma then coated with 0.1% Chitosan solution 2	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Argon plasma then coated with 0.1% Chitosan solution 3	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Ammonia plasma then coated with 0.1% Chitosan solution 1	140000	0.00	0.00	0.00	0.00

Solanyl Flexibilitis treated with Ammonia plasma then coated with 0.1% Chitosan solution 2	140000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Ammonia plasma then coated with 0.1% Chitosan solution 3	140000	0.00	0.00	0.00	0.00

Day	0	8	15	22	29
Date	03/08/2005	11/08/2005	18/08/2005	25/08/2005	01/09/2005
Solanyl Flexibilitis 1	235000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis 2	235000	0.00	0.00	0.00	21.00
Solanyl Flexibilitis 3	235000	0.00	0.00	7.00	0.00
Solanyl Flexibilitis treated with Argon plasma 1	235000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Argon plasma 2	235000	0.00	0.50	27.00	19.00
Solanyl Flexibilitis treated with Argon plasma 3	235000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Ammonia plasma 1	235000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Ammonia plasma 2	235000	2.00	16.00	24.00	30.00
Solanyl Flexibilitis treated with Ammonia plasma 3	235000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis extruded with 2% Chitosan powder (w/w) 1	235000	0.00	0.00	15.00	30.00
Solanyl Flexibilitis extruded with 2% Chitosan powder (w/w) 2	235000	0.00	1.75	15.00	30.00
Solanyl Flexibilitis extruded with 2% Chitosan powder (w/w) 3	235000	0.00	0.00	0.00	0.00

Solanyl Flexibilitis coated with 0.1% Chitosan solution 1	235000	0.00	1.00	0.00	0.00
Solanyl Flexibilitis coated with 0.1% Chitosan solution 2	235000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis coated with 0.1% Chitosan solution 3	235000	0.00	7.00	7.00	17.50
Solanyl Flexibilitis treated with Argon plasma then coated with 0.1% Chitosan solution 1	235000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Argon plasma then coated with 0.1% Chitosan solution 2	235000	0.00	0.00	0.00	4.00
Solanyl Flexibilitis treated with Argon plasma then coated with 0.1% Chitosan solution 3	235000	0.00	6.00	6.00	26.00
Solanyl Flexibilitis treated with Ammonia plasma then coated with 0.1% Chitosan solution 1	235000	0.00	4.00	2.00	21.00
Solanyl Flexibilitis treated with Ammonia plasma then coated with 0.1% Chitosan solution 2	235000	0.00	0.25	5.00	30.00
Solanyl Flexibilitis treated with Ammonia plasma then coated with 0.1% Chitosan solution 3	235000	0.00	1.00	16.00	30.00

Day	0	8	15	22	29
Date	25/08/2005	02/09/2005	09/09/2005	16/09/2005	23/09/2005
Solanyl Flexibilitis 1	190000	14.50	24.00	30.00	30.00
Solanyl Flexibilitis 2	190000	0.00	6.00	0.00	0.00
Solanyl Flexibilitis 3	190000	n/a	n/a	n/a	n/a
Solanyl Flexibilitis treated with Argon plasma 1	190000	n/a	n/a	n/a	n/a
Solanyl Flexibilitis treated with Argon plasma 2	190000	n/a	n/a	n/a	n/a

Solanyl Flexibilitis treated with Argon plasma 3	190000	n/a	n/a	n/a	n/a
Solanyl Flexibilitis treated with Ammonia plasma 1	190000	n/a	n/a	n/a	n/a
Solanyl Flexibilitis treated with Ammonia plasma 2	190000	n/a	n/a	n/a	n/a
Solanyl Flexibilitis treated with Ammonia plasma 3	190000	n/a	n/a	n/a	n/a
Solanyl Flexibilitis extruded with 2% Chitosan powder (w/w) 1	190000	n/a	n/a	n/a	n/a
Solanyl Flexibilitis extruded with 2% Chitosan powder (w/w) 2	190000	n/a	n/a	n/a	n/a
Solanyl Flexibilitis extruded with 2% Chitosan powder (w/w) 3	190000	n/a	n/a	n/a	n/a
Solanyl Flexibilitis coated with 0.1% Chitosan solution 1	190000	0.00	0.00	8.00	10.00
Solanyl Flexibilitis coated with 0.1% Chitosan solution 2	190000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis coated with 0.1% Chitosan solution 3	190000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Argon plasma then coated with 0.1% Chitosan solution 1	190000	0.00	0.00	0.00	0.00
Solanyl Flexibilitis treated with Argon plasma then coated with 0.1% Chitosan solution 2	190000	0.00	0.00	13.25	8.00
Solanyl Flexibilitis treated with Argon plasma then coated with 0.1% Chitosan solution 3	190000	3.00	30.00	30.00	30.00
Solanyl Flexibilitis treated with Ammonia plasma then coated with 0.1% Chitosan solution 1	190000	0.00	0.00	0.00	0.00

Solanyl Flexibilitis treated with Ammonia plasma then coated with 0.1% Chitosan solution 2	190000	2.00	28.00	30.00	30.00
Solanyl Flexibilitis treated with Ammonia plasma then coated with 0.1% Chitosan solution 3	190000	0.00	0.00	0.00	0.00

Poly-L-Lactic acid Data

Day	0	8	15	22	29
Date	16/07/2005	24/07/2005	31/07/2005	07/08/2005	14/08/2005
Poly-L-Lactic acid 1	100000	0.00	17.50	17.50	30.00
Poly-L-Lactic acid 2	100000	2.00	0.00	0.00	0.00
Poly-L-Lactic acid 3	100000	0.50	0.00	0.00	0.00
Poly-L-Lactic acid treated with Argon plasma 1	100000	0.00	0.00	0.00	0.00
Poly-L-Lactic acid treated with Argon plasma 2	100000	0.00	0.00	0.00	0.00
Poly-L-Lactic acid treated with Argon plasma 3	100000	6.00	30.00	30.00	30.00
Poly-L-Lactic acid treated with Ammonia plasma 1	100000	0.00	2.50	0.00	0.00
Poly-L-Lactic acid treated with Ammonia plasma 2	100000	0.00	0.00	0.00	0.00
Poly-L-Lactic acid treated with Ammonia plasma 3	100000	3.00	3.00	30.00	30.00

Day	0	8	15	22	29
Date	21/08/2005	29/08/2005	05/09/2005	12/09/2005	19/09/2005
Poly-L-Lactic acid 1		Can’t measure. Cells growing on	Cell attachment, can't measure.	Cell attachment, can't measure.	Cell attachment at both ends. Can't

		tissue culture flask, + cells growing on capillary tube. Media remained red	Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	measure, probably all the way. Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange
Poly-L-Lactic acid 2		Can’t measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	Cell attachment, can't measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	Cell attachment, can't measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	Cell attachment at both ends. Can't measure, probably all the way. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange
Poly-L-Lactic acid 3		Can’t measure.	Cell attachment,	Cell attachment,	Cell attachment

		Cells growing on tissue culture flask, + cells growing on capillary tube. Media remained red	can't measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	can't measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	at both ends. Can't measure, probably all the way. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange
Poly-L-Lactic acid treated with Argon plasma 1		Can’t measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	Cell attachment, can't measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	Cell attachment, can't measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	Cell attachment at both ends. Can't measure, probably all the way. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange

Poly-L-Lactic acid treated with Argon plasma 2		0	0	0	0
Poly-L-Lactic acid treated with Argon plasma 3		Can’t measure Cells growing on tissue culture flask, + cells growing on capillary tube. Media remained red	Few Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange.	Cell attachment, can't measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	Cell attachment at one end, can't measure + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange
Poly-L-Lactic acid treated with Ammonia plasma 1		Cell attachment. No cells growing on capillary tube or tissue culture flask. Media remained red	Cell attachment, cells growing on capillary tube. Media turned orange	Cell attachment, can't measure. Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Cell attachment at one end, possibly the other, can't measure + cells growing on capillary tube + cells growing on tissue culture flask. Media turned

					orange
Poly-L-Lactic acid treated with Ammonia plasma 2		0	0	0	0
Poly-L-Lactic acid treated with Ammonia plasma 3		Cell attachment. No cells growing on capillary tube or tissue culture flask. Media remained red	Cell attachment, cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Cell attachment, can't measure, cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Cell attachment at one end, possibly the other, can't measure + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange

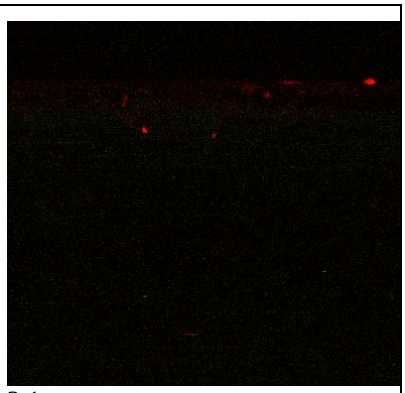
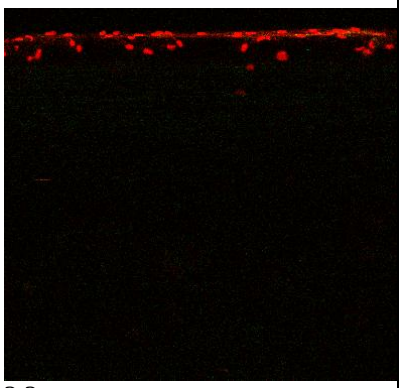
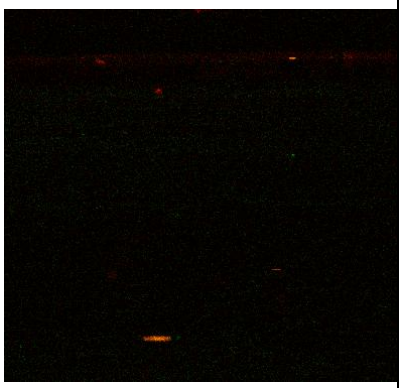
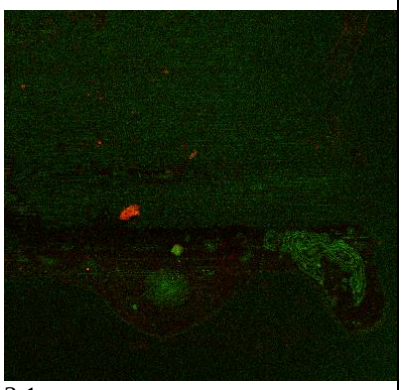
Day	0	8	15	22	29
Date	25/08/2005	02/09/2005	09/09/2005	16/09/2005	23/09/2005
Poly-L-Lactic acid 1	240000	Few cells growing on capillary tube. sample came off	Cell attachment. Free floating. Possibly confluent. Cells growing on tissue culture flask, + cells	Cell attachment, can't measure. Cells growing on tissue culture flask, + cells growing on capillary	Can’t measure. (Free floating. Both ends have cells. Few cells growing on tissue culture flask, + cells

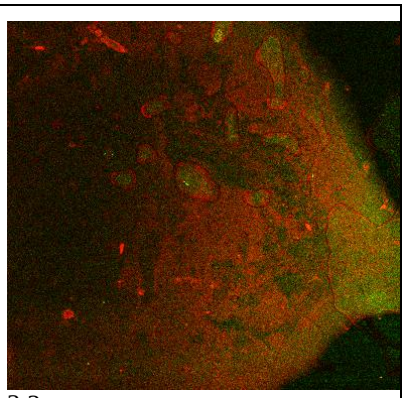
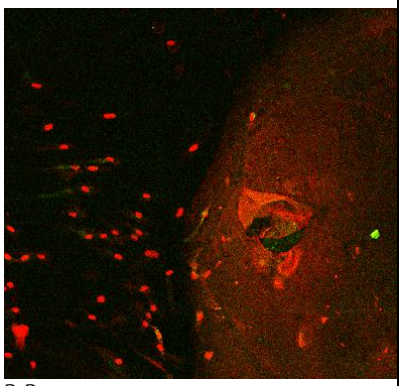
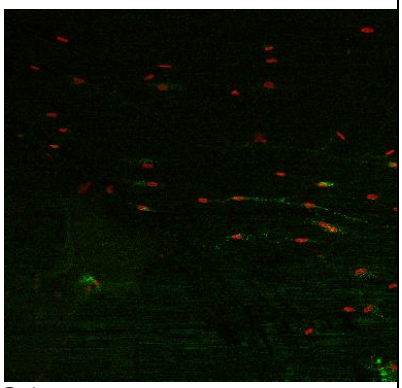
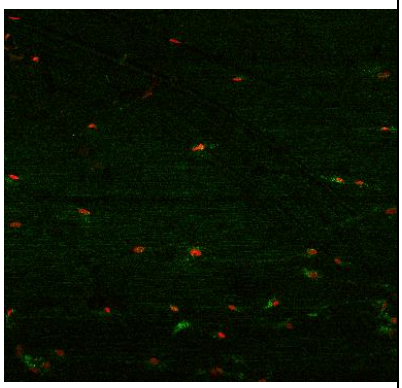
			growing on capillary tube. Media turned orange	tube. Media turned orange	growing on capillary tube. Media turned orange
Poly-L-Lactic acid 2	240000	Few Cells growing on tissue culture flask. No cells growing on capillary tube.	Cell attachment, free floating. Cells growing on capillary tube. Media remained red	Cell attachment, can't measure. Cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	Cells on one end contracting sample. Can't measure. Probably not on other end. Cells growing on tissue culture flask. Media turned orange
Poly-L-Lactic acid 3	240000	0	0	0	0
Poly-L-Lactic acid treated with Argon plasma 1	240000	0	0	0	0
Poly-L-Lactic acid treated with Argon plasma 2	240000	0	0	0	0
Poly-L-Lactic acid treated with Argon plasma 3	240000	0	0	0	0
Poly-L-Lactic acid treated with Ammonia plasma 1	240000	0	0	0	0
Poly-L-Lactic acid treated with Ammonia plasma 2	240000	0	0	0	0
Poly-L-Lactic acid treated with Ammonia plasma 3	240000	0	0	0	0

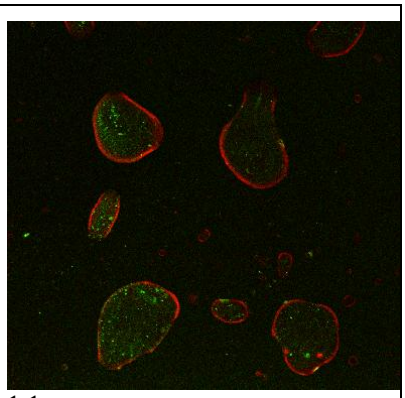
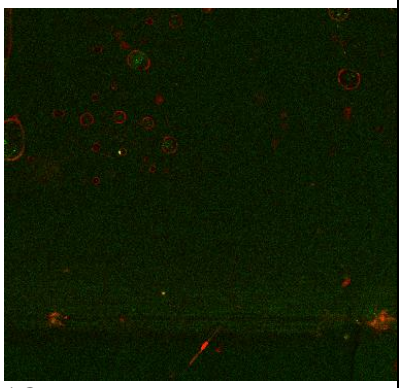
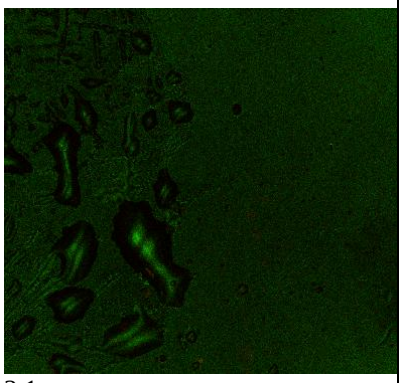
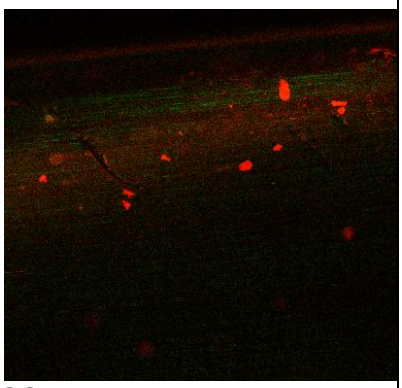
C.2 Fluorescence images

Below, a list of tissue culture samples with the measurements from the last day of measuring growth along the sample, with corresponding fluorescence images and descriptions from the last day of each sample. Where there are no fluorescence images, there was either nothing to see, or no good image could be obtained.

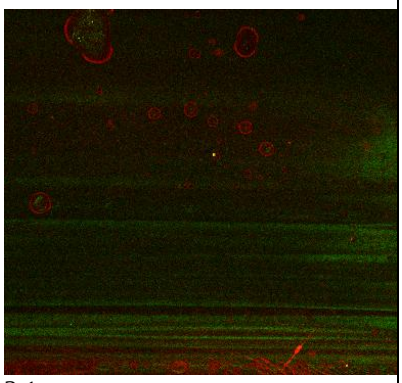
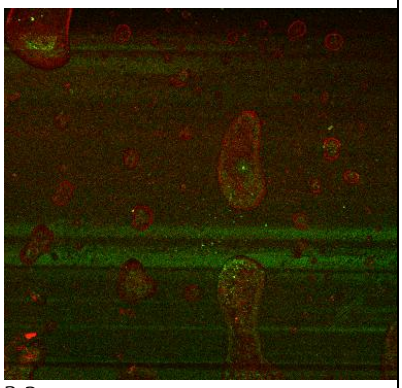
	Sample	Growth measured on previous day and observations	Notes recorded during fluorescence measurement	Fluorescence microscopy images (and image number)
Day	0	29	30	
Date	20/06/2005	19/07/2005	20/07/2005	
Polypropylene 1		0.0 mm	no visible cells	No image
Polypropylene 2		0.0 mm	no visible cells	No image
Polypropylene 3		0.0 mm	no visible cells	No image
Polypropylene + Argon 1		0.0 mm	no visible cells	No image
Polypropylene + Argon 2		0.0 mm	no visible cells	No image
Polypropylene + Argon 3		0.0 mm	no visible cells	No image
Polypropylene + ammonia 1		0.0 mm	no visible cells	No image
Polypropylene + ammonia 2		0.0 mm	no visible cells	No image
Polypropylene + ammonia 3		0.0 mm	no visible cells	No image
Polypropylene + Chitosan 1		0.0 mm	no visible cells	No image

Polypropylene + Chitosan 2	2.8 mm + cells growing on capillary tube		 2.1  2.2  2.3
Polypropylene + Chitosan 3	5 mm + cells growing on capillary tube		 3.1

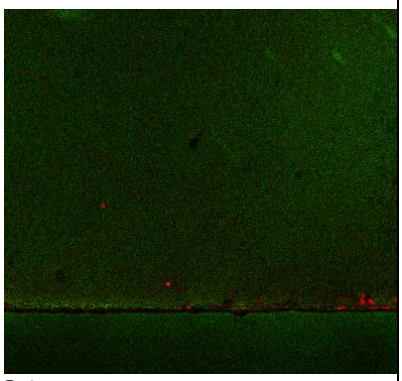
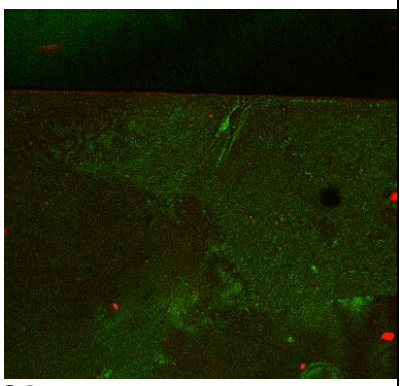
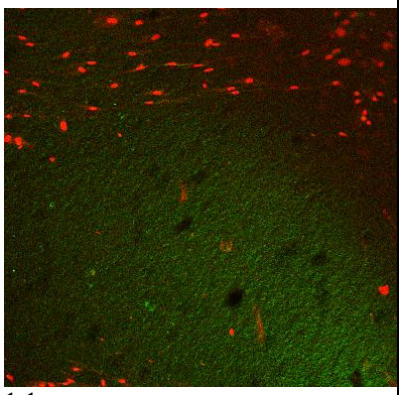
			 3.2  3.3  3.4  3.5

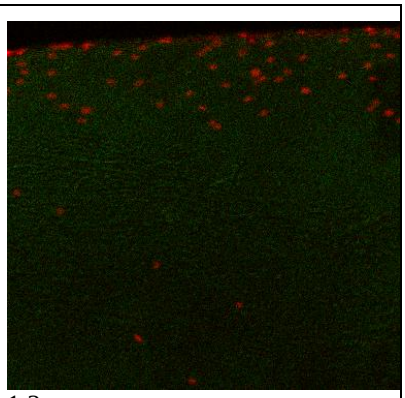
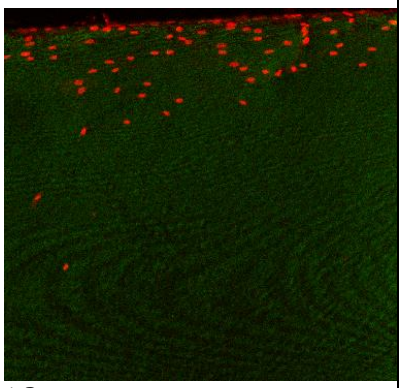
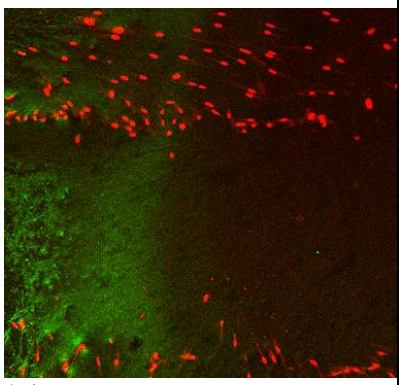
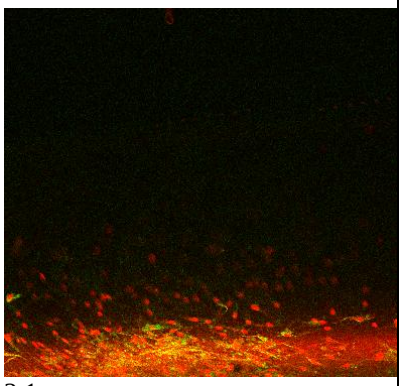
Polypropylene + Argon + Chitosan 1	0.0 mm		 1.1  1.2
Polypropylene + Argon + Chitosan 2	0.0 mm		 2.1  2.2
Polypropylene +	0.0 mm	no visible cells	No image

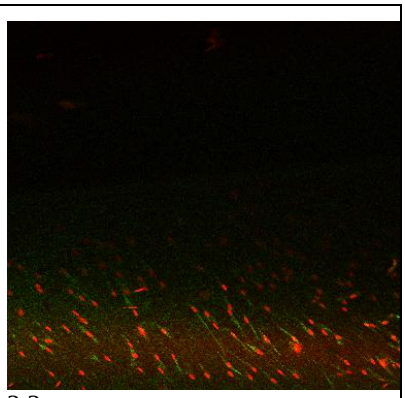
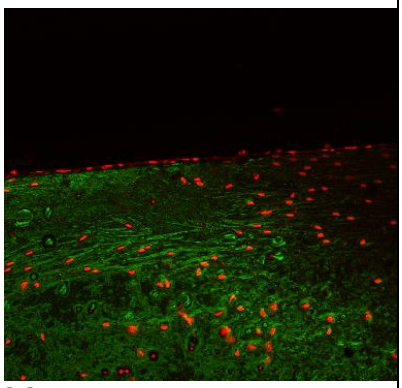
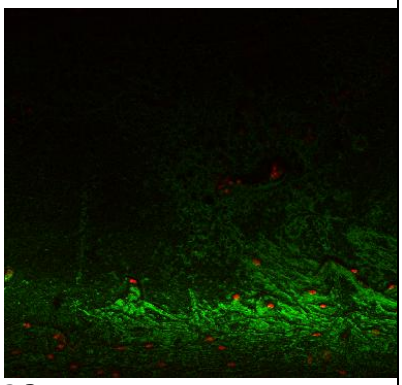
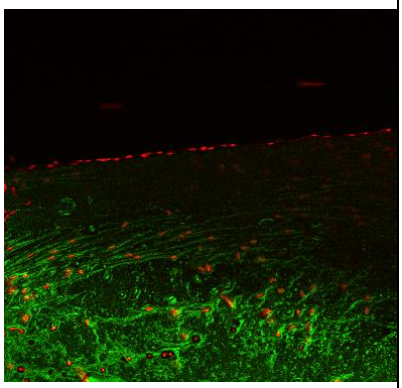
Argon + Chitosan 3			
Polypropylene + ammonia + Chitosan 1	0.0 mm	no visible cells	No image
Polypropylene + ammonia + Chitosan 2	0.0 mm	no visible cells	No image
Polypropylene + ammonia + Chitosan 3	0.0 mm	no visible cells	No image

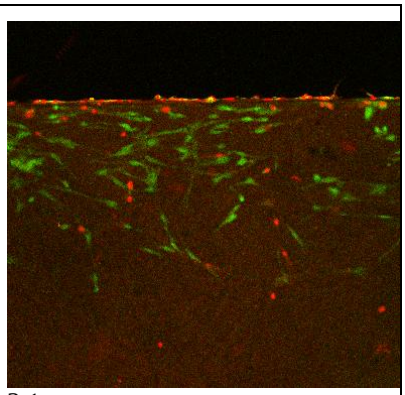
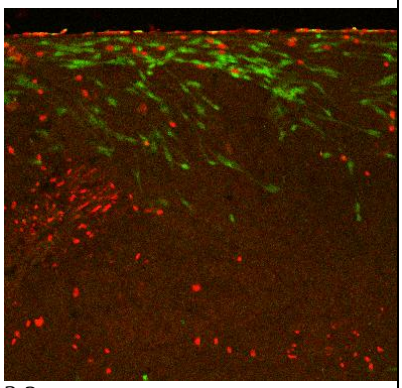
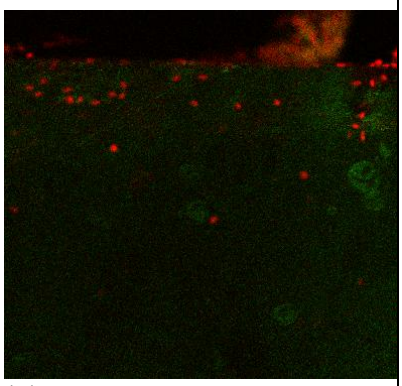
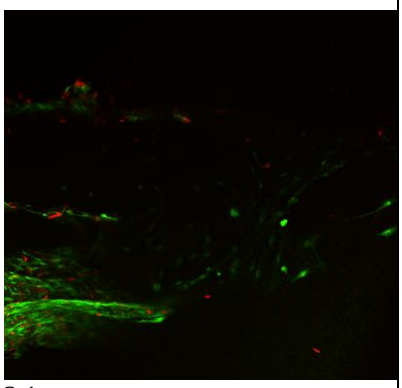
Day	0	29.0		
Date	20/06/2005	19/07/2005	20/07/2005	
Solanyl 1		0.0 mm	no visible cells	No image
Solanyl 2		0.0 mm	no visible cells	No image
Solanyl 3		0.0 mm	no visible cells	 3.1  3.2
Solanyl + Argon 1		0.0 mm	no visible cells	No image
Solanyl + Argon 2		0.0 mm	no visible cells	No image
Solanyl + Argon 3		0.0 mm	no visible cells	No image
Solanyl + ammonia 1		0.0 mm	no visible cells	No image
Solanyl + ammonia 2		0.0 mm	no visible cells	No image
Solanyl + ammonia 3		0.0 mm	no visible cells	No image
Solanyl 2% Chitosan 1		0.0 mm	no visible cells	No image
Solanyl 2% Chitosan 2		0.0 mm	no visible cells	No image
Solanyl 2% Chitosan		0.0 mm	no visible cells	No image

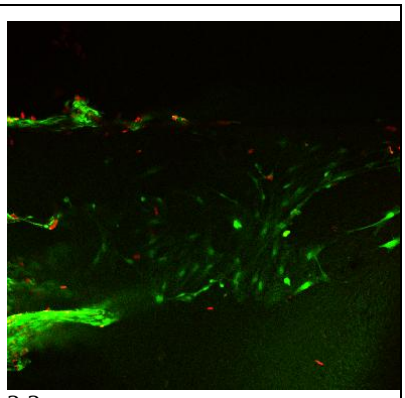
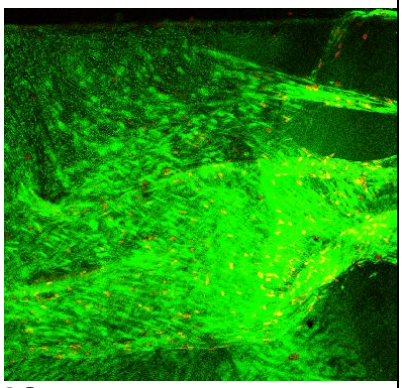
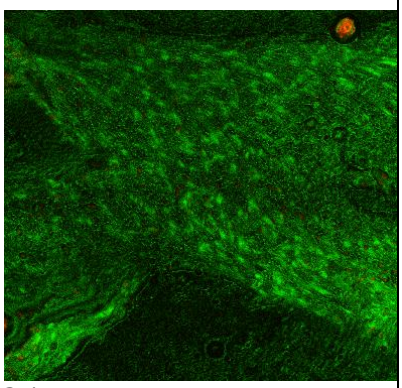
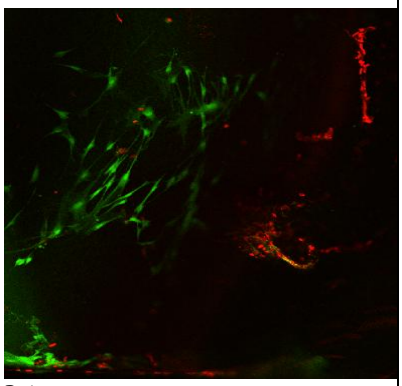
3			
Solanyl + Chitosan 1	0.0 mm	no visible cells	No image
Solanyl + Chitosan 2	0.0 mm	no visible cells	No image
Solanyl + Chitosan 3	2 mm + cells growing on capillary tube	no visible cells	No image
Solanyl + Argon + Chitosan 1	0.0 mm	no visible cells	No image
Solanyl + Argon + Chitosan 2	0.0 mm	no visible cells	No image
Solanyl + Argon + Chitosan 3	0.0 mm	no visible cells	No image
Solanyl + ammonia + Chitosan 1	0.0 mm	no visible cells	No image
Solanyl + ammonia + Chitosan 2	0.0 mm	no visible cells	No image
Solanyl + ammonia + Chitosan 3	0.0 mm	no visible cells	No image

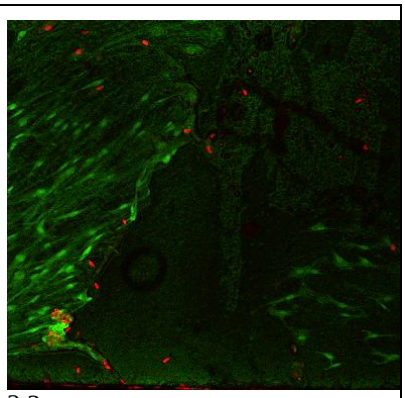
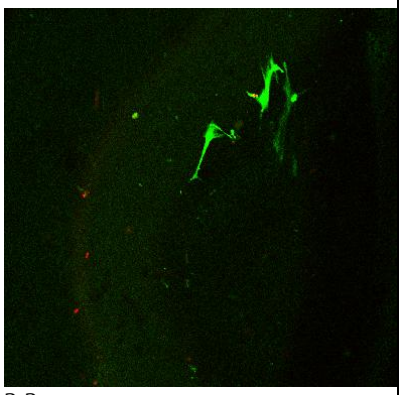
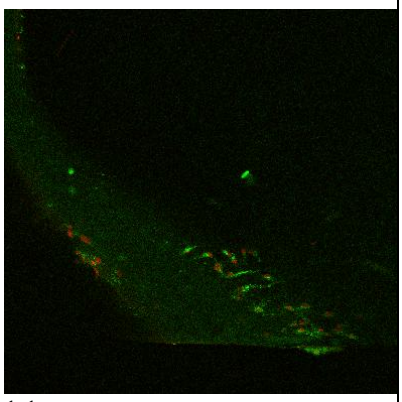
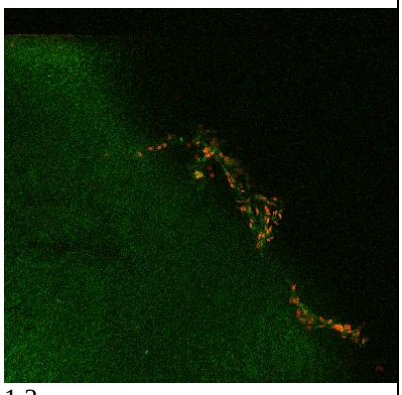
Day	0.0	29.0	31.0	
Date	14/07/2005	12/08/2005	14/08/2005	
Polyurethane 1		0 mm Media remained red	no visible cells	No image
Polyurethane 2		14 mm + cells growing on capillary tube. Media turned orange.	2 pictures	 3.1  3.2
Polyurethane 3		0 mm Media remained red	no visible cells	No image
Polyurethane + Argon 1		30 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	4 pictures	 1.1

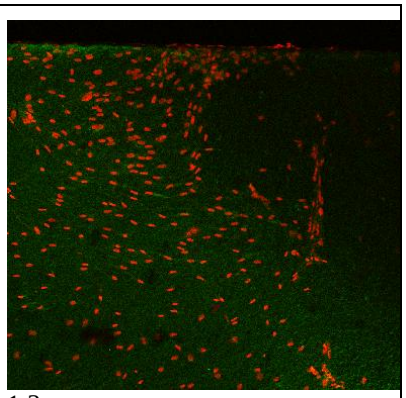
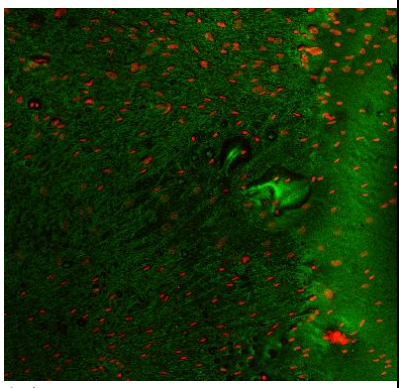
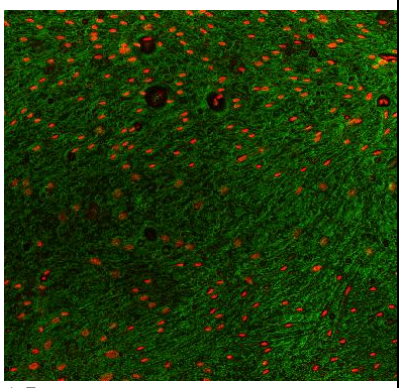
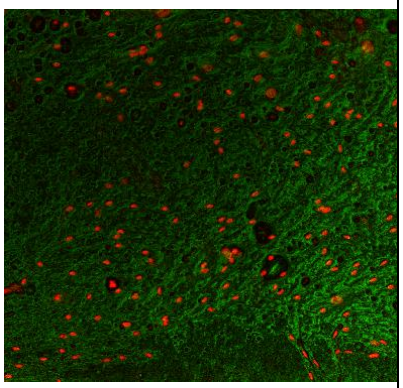
			 1.2  1.3  1.4
Polyurethane + Argon 2	30 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	5 pictures	 2.1

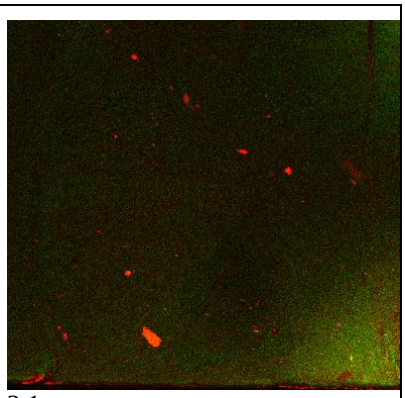
			 2.2  2.2  2.3  2.4

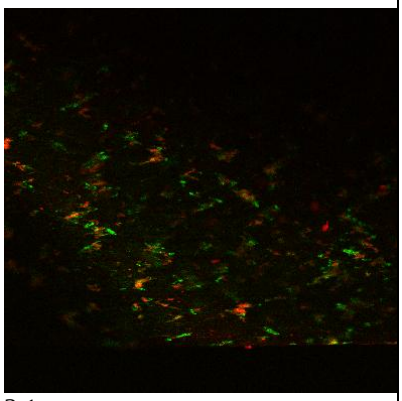
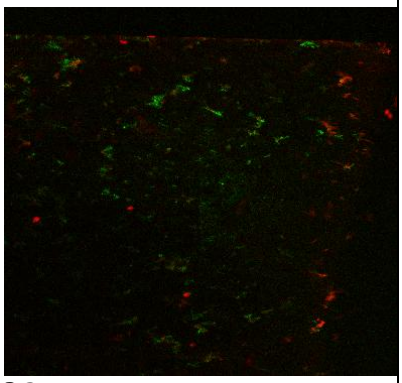
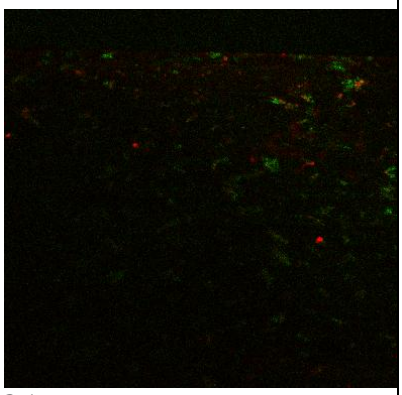
Polyurethane + Argon 3	10 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	cells 1/4 way up	 3.1  3.2
Polyurethane + ammonia 1	26 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.		 1.1
Polyurethane + ammonia 2	16 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.		 2.1

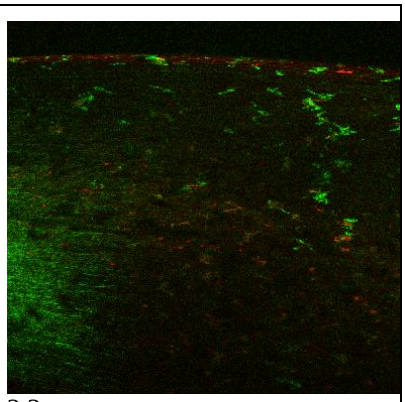
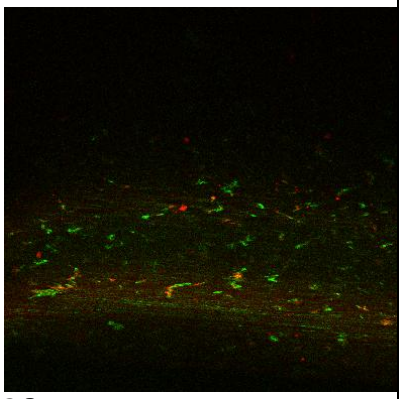
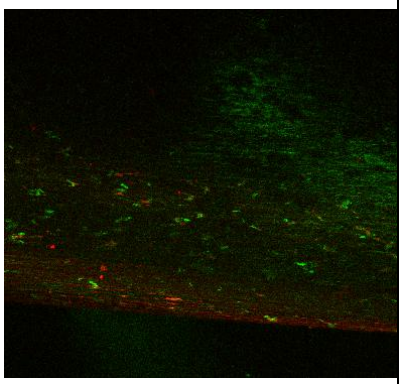
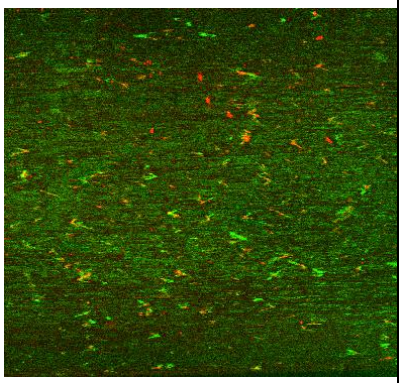
			 2.2  2.3  2.4
Polyurethane + ammonia 3	30 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.		 3.1

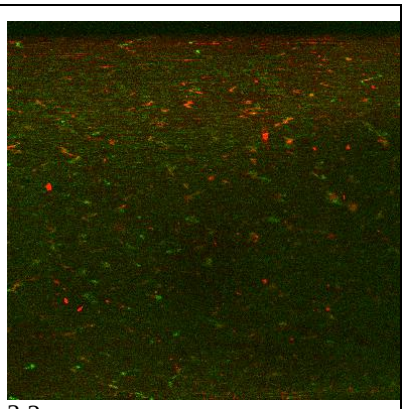
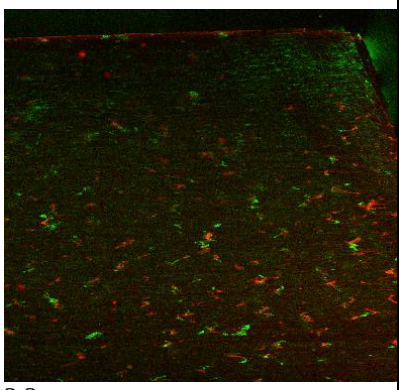
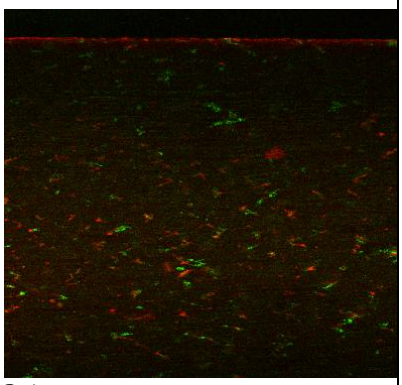
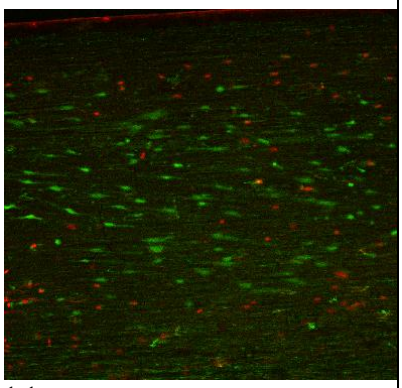
			 3.2  3.3
Polyurethane + Argon + Chitosan powder 1	30 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.		 1.1  1.2

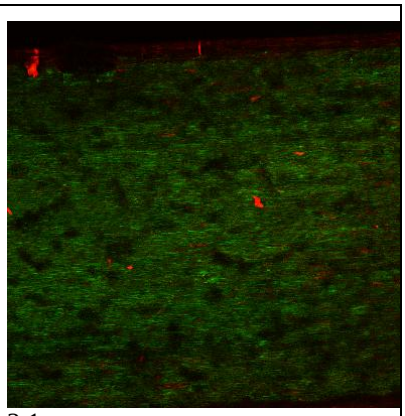
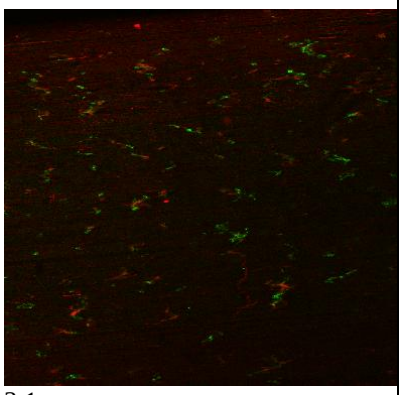
			 1.3  1.4  1.5  1.6

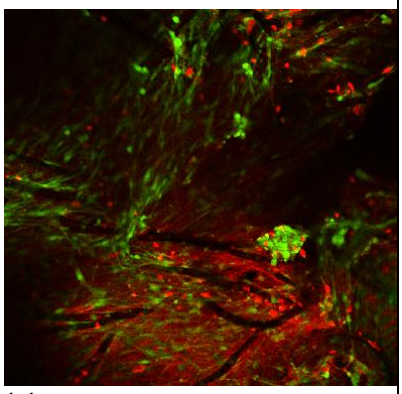
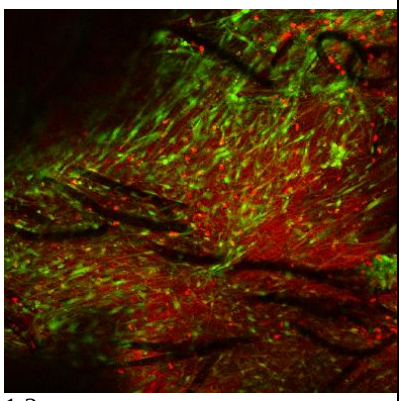
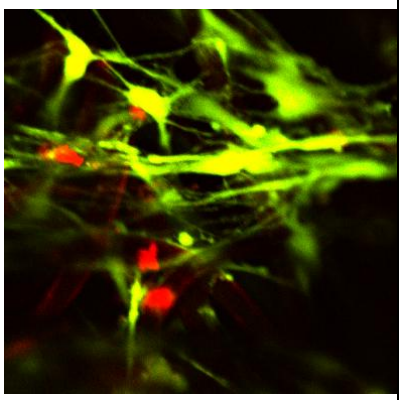
Polyurethane + Argon + Chitosan powder 2	0 mm Media remained red	no visible cells	 2.1
Polyurethane + Argon + Chitosan powder 3	2.25 mm + cells growing on capillary tube Media remained red	Growth ¼ along the length of sample?	No image

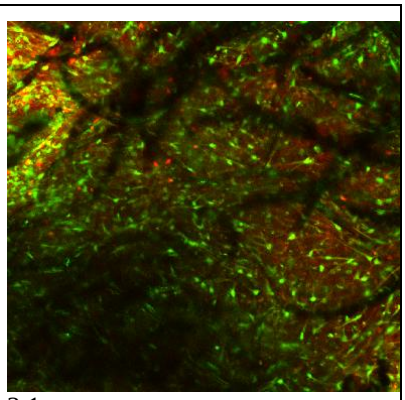
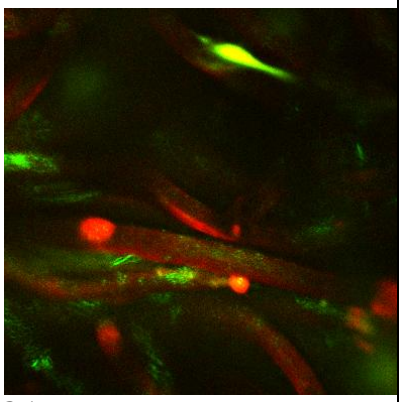
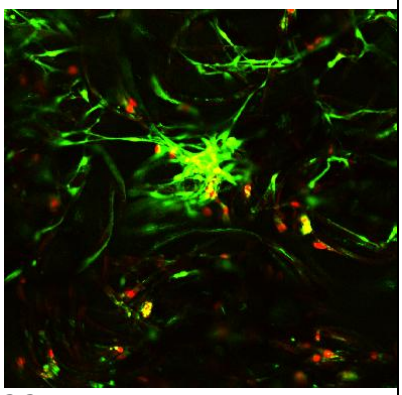
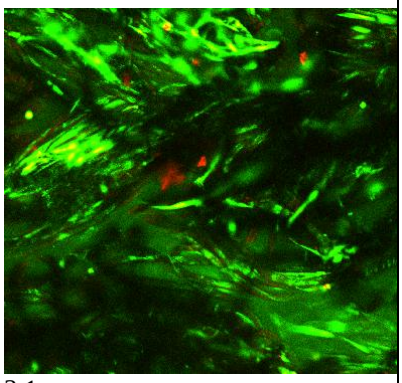
Day	0.0	29.0	31.0	
Date	14/07/2005	12/08/2005	14/07/2005	
polycaprolactone 1		0 mm Media remained red	no visible cells	No image
polycaprolactone 2		0 mm Media remained red	no visible cells	No image
polycaprolactone 3		0.4 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	one end of sample	 3.1  3.2
polycaprolactone + Argon 1		0 mm Media remained red	no visible cells	No image
polycaprolactone + Argon 2		0 mm Media remained red	Growth at both ends. 2.1= one end	 2.1

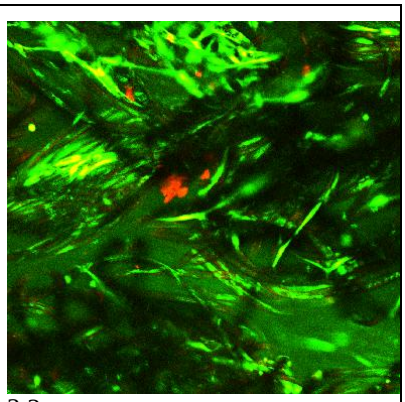
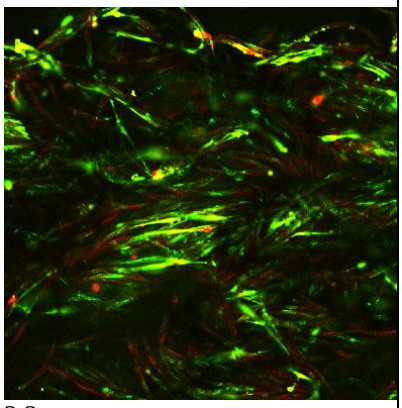
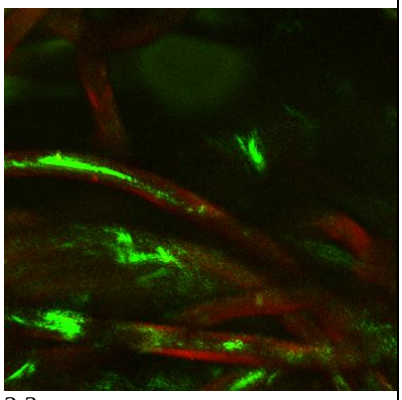
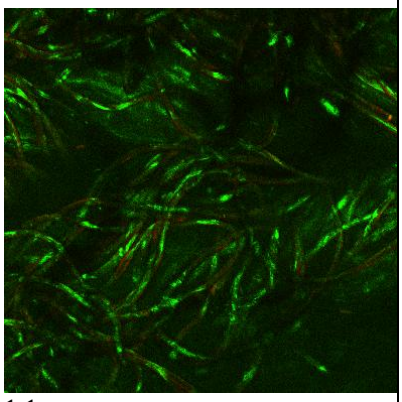
			 2.2  2.3  2.4
polycaprolactone + Argon 3	0 mm Media remained red	Cells span 1/3 of sample from one end and ½ way along sample from other end	 3.1

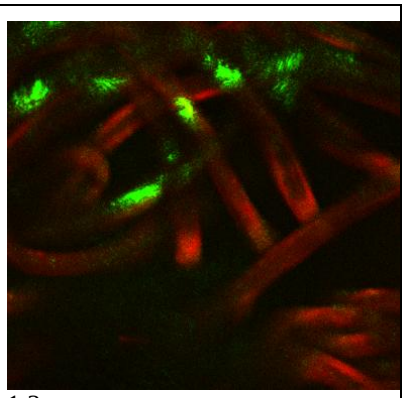
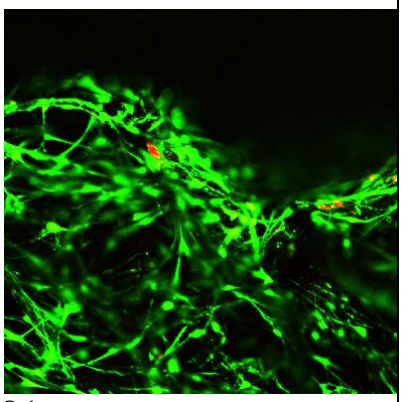
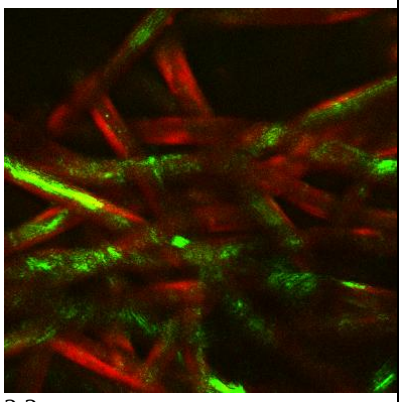
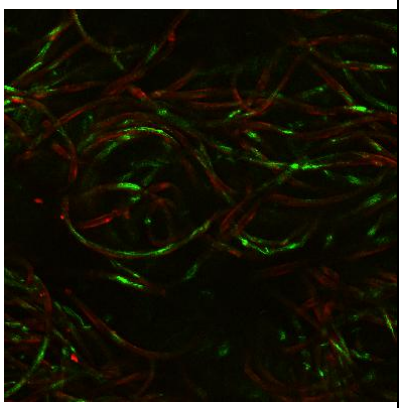
			 3.2  3.3  3.4
polycaprolactone + ammonia 1	6 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	1/4 to 1/2	 1.1

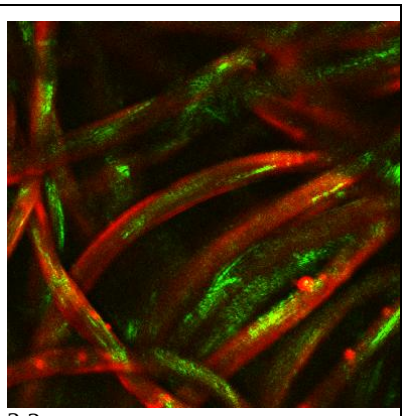
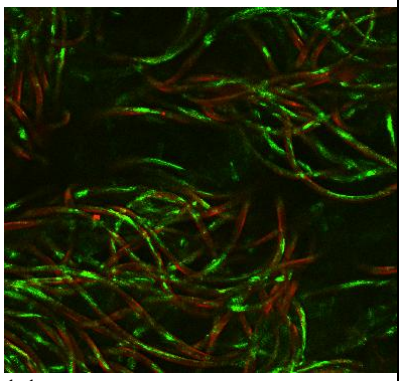
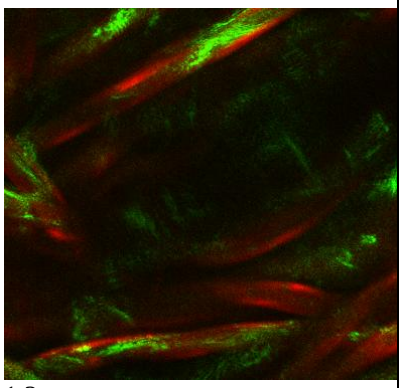
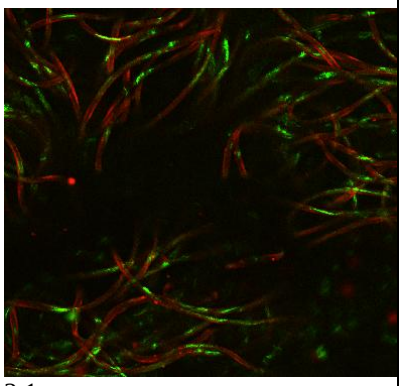
polycaprolactone + ammonia 2	0.0 mm Media remained red	no visible cells	 2.1
polycaprolactone + ammonia 3	0 mm Media remained red	1/3 to 1/2	 3.1

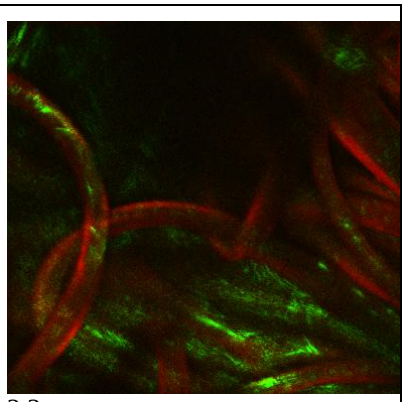
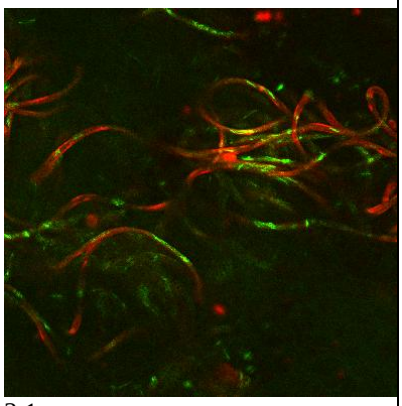
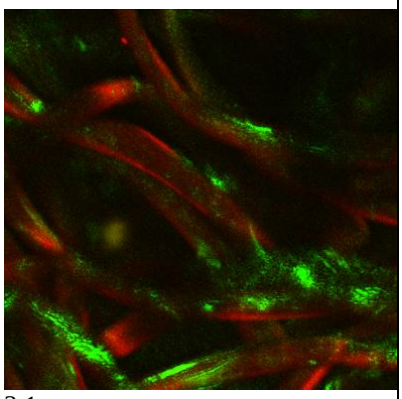
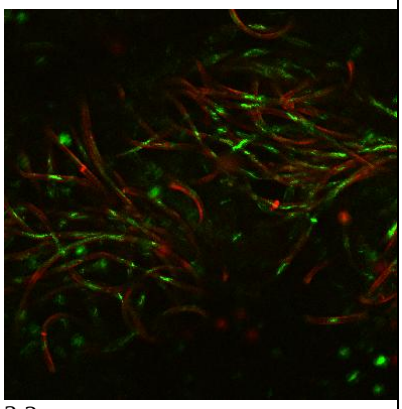
Day	0.0	29.0	30.0	
Date	16/07/2005	14/08/2005	15/08/2005	
Polyester 1		6 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Cells visible all the way along sample or fibres fluorescing	 1.1  1.2  1.2 zoom

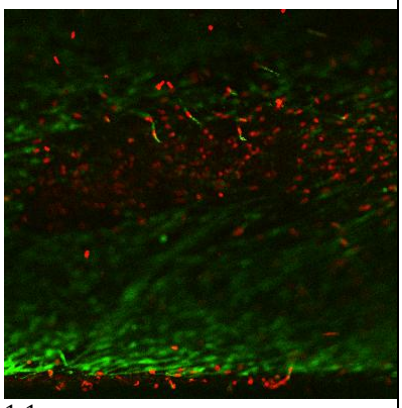
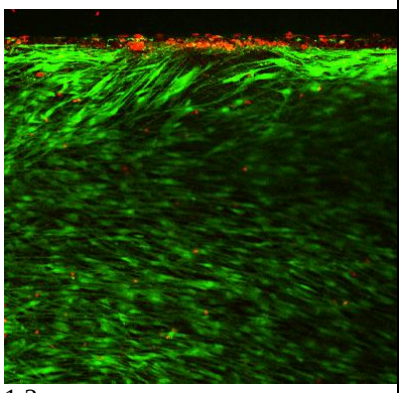
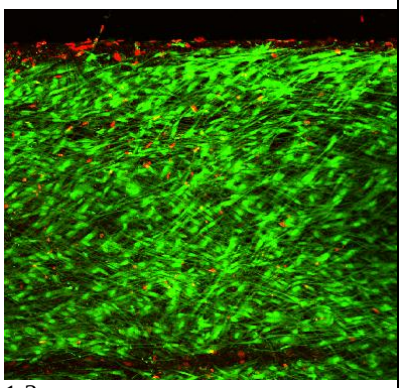
Polyester 2	11 mm dense, 30 mm total + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Cells visible all the way along sample or fibres fluorescing	 2.1  2.1 zoom  2.2
Polyester 3	8 mm dense, 30 mm total + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Cells visible all the way along sample or fibres fluorescing	 3.1

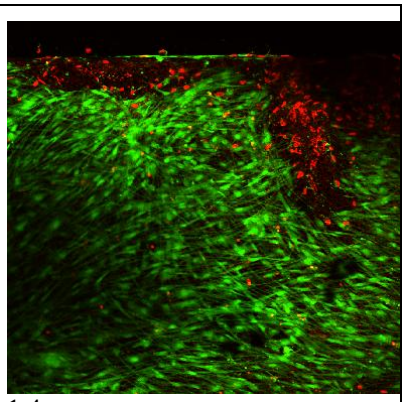
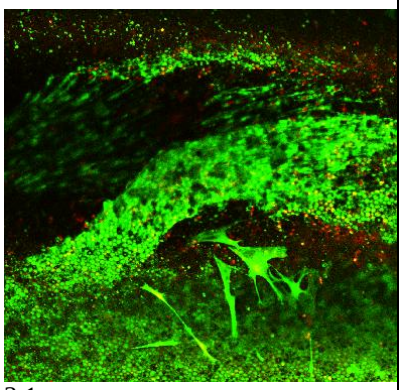
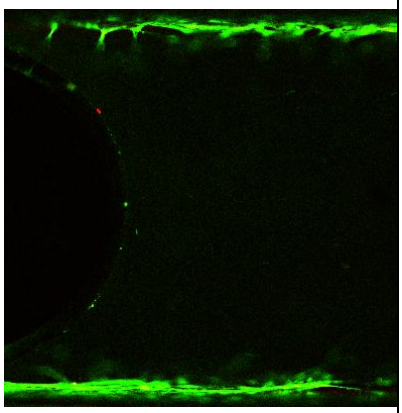
			 3.2  3.3  3.3 zoom
Polyester + Argon 1	0 mm Media remained red	Cells visible all the way along sample or fibres fluorescing	 1.1

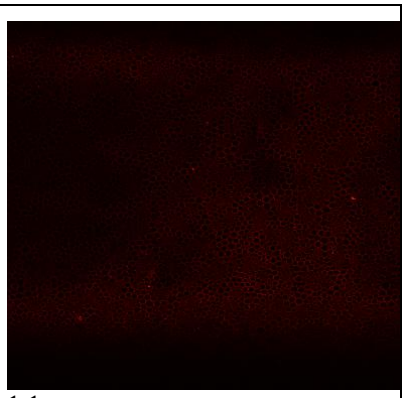
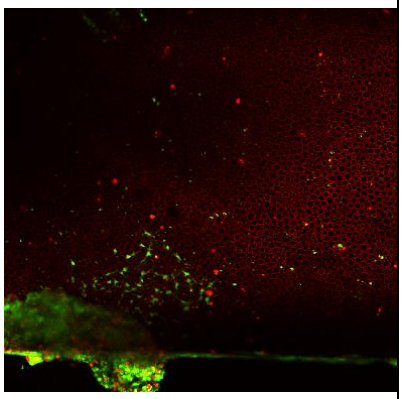
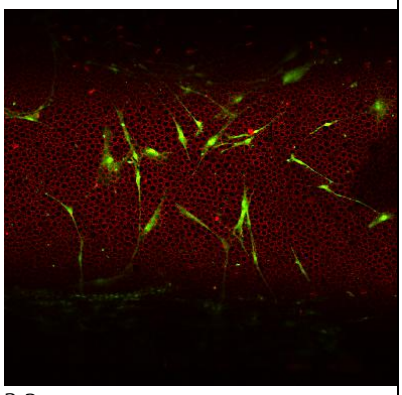
			 1.2 zoom
Polyester + Argon 2	6 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Cells visible all the way along sample or fibres fluorescing	 2.1  2.2 zoom
Polyester + Argon 3	0 mm Media remained red	Cells visible all the way along sample or fibres fluorescing	 3.1

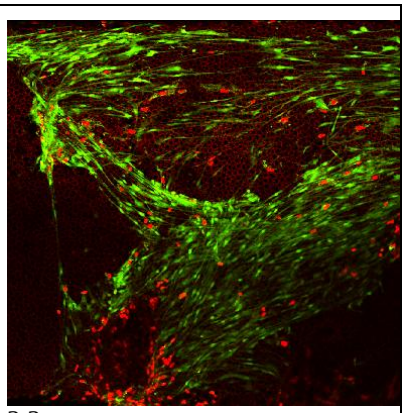
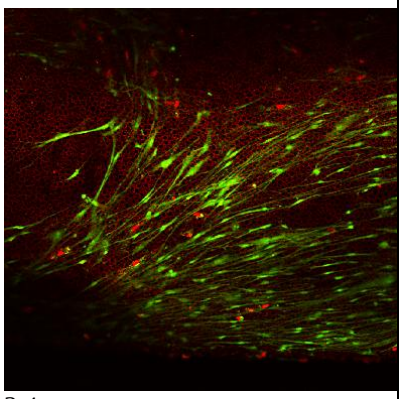
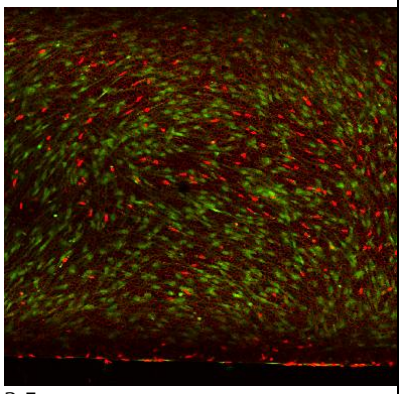
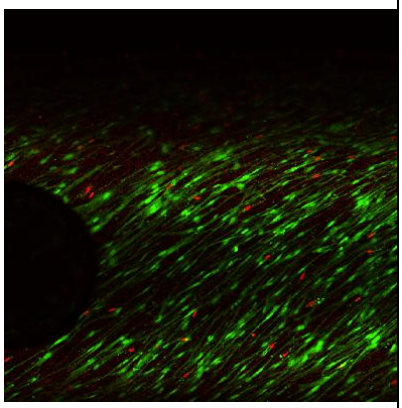
			 3.2 zoom
Polyester + ammonia 1	0 mm Media remained red	Cells visible all the way along sample or fibres fluorescing	 1.1  1.2 zoom
Polyester + ammonia 2	0 mm Media remained red	Cells visible all the way along sample or fibres fluorescing	 2.1

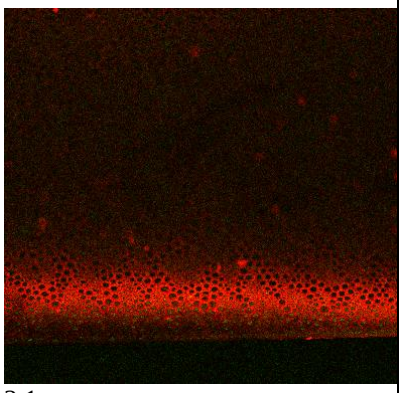
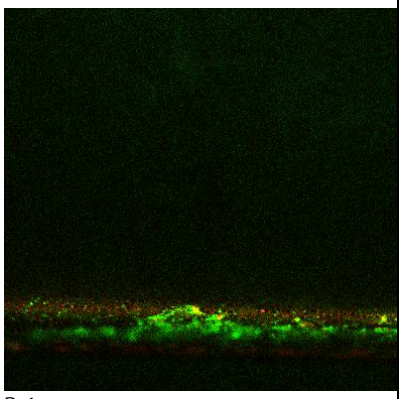
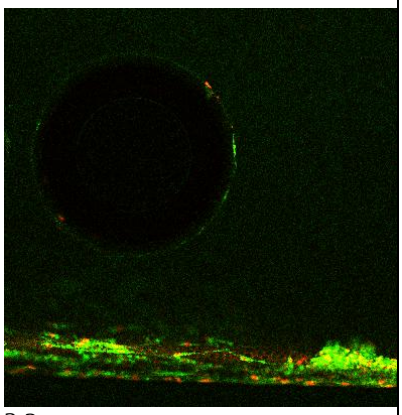
			 2.2 zoom
Polyester + ammonia 3	0 mm Media remained red	Cells visible all the way along sample or fibres fluorescing	 3.1  3.1 zoom  3.2

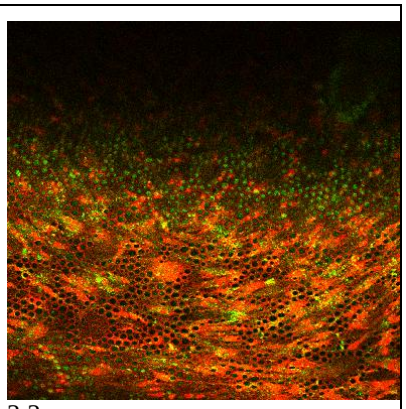
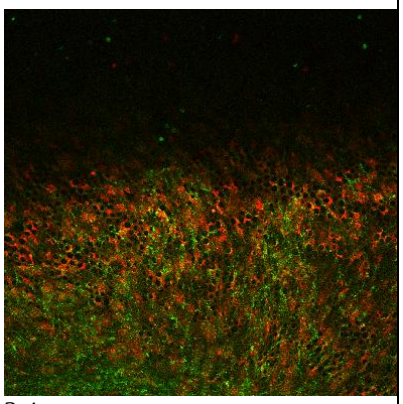
Day	0.0	29.0	30.0	
Date	16/07/2005	14/08/2005	15/08/2005	
Polylactic acid 1		Cells growing on capillary tube + cells growing on tissue culture flask, possibly all the way.	Cells visible all the way along sample	 1.1  1.2  1.3

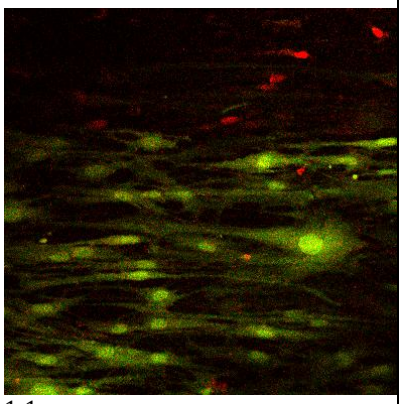
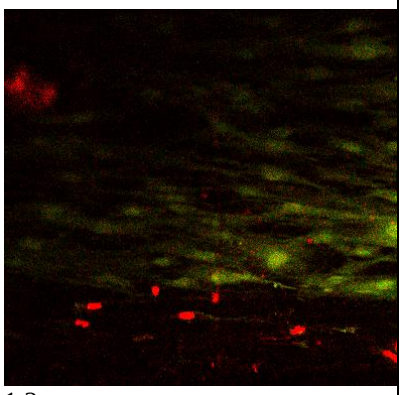
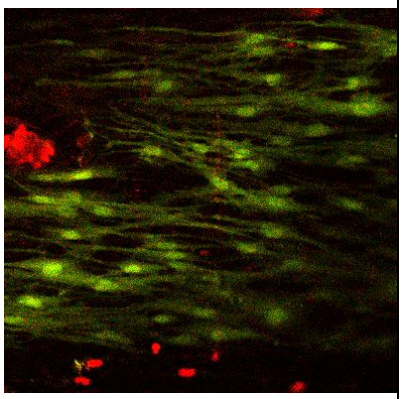
			 1.4
Polylactic acid 2	Cells growing on capillary tube + cells growing on tissue culture flask, possibly all the way.	no visible cells	No image
Polylactic acid 3	Cells growing on capillary tube + cells growing on tissue culture flask, possibly all the way.	0.7	 3.1  3.2

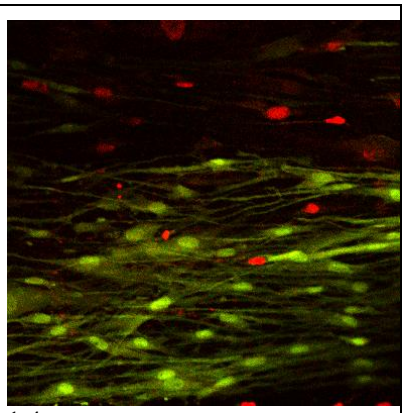
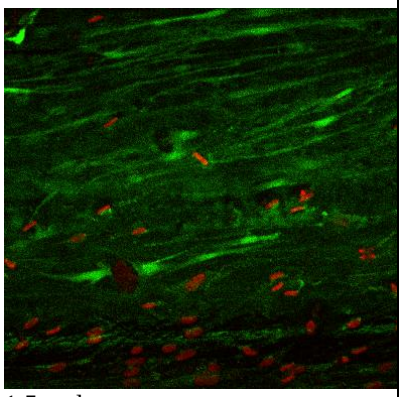
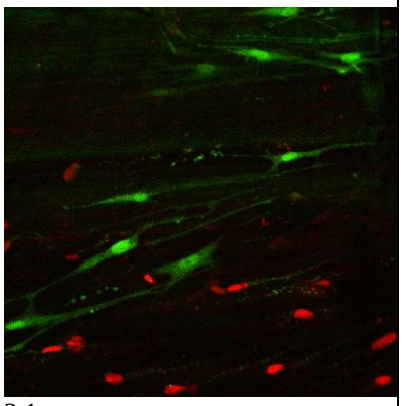
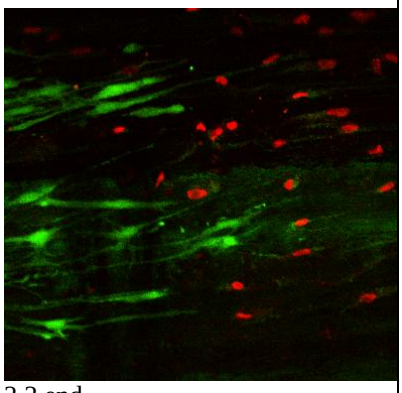
Polylactic acid + Argon 1	0 mm Media remained red	no visible cells	 1.1
Polylactic acid + Argon 2	0 mm Media remained red	no visible cells	No image
Polylactic acid + Argon 3	Cells growing on capillary tube + cells growing on tissue culture flask, possibly all the way.	Cells visible all the way along sample	 3.1  3.2

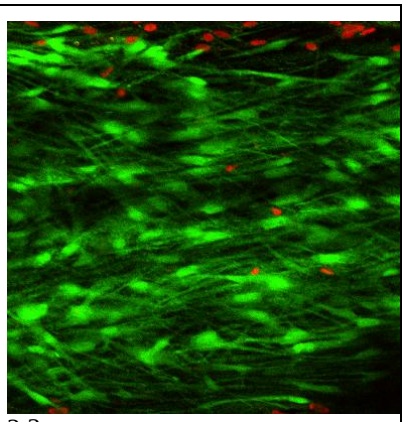
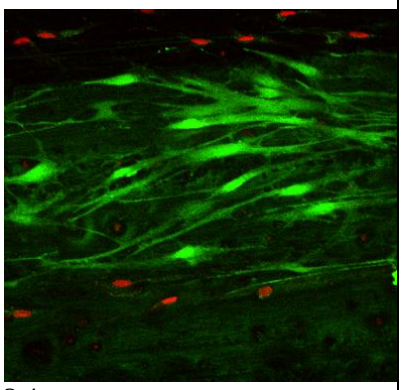
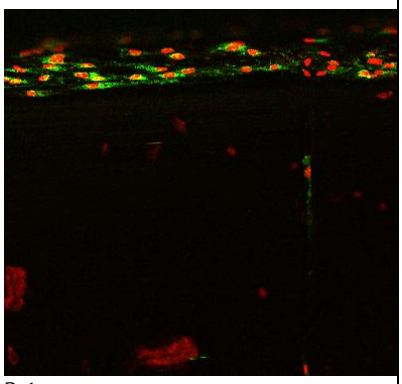
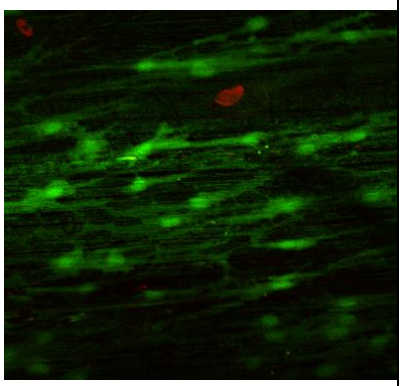
			 3.3  3.4  3.5  3.6

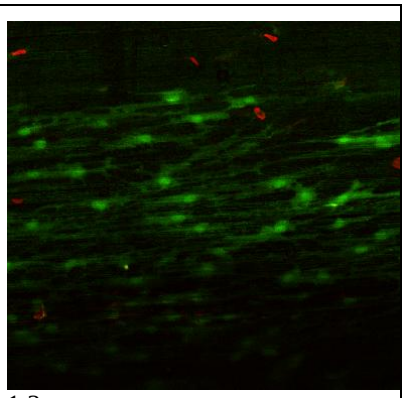
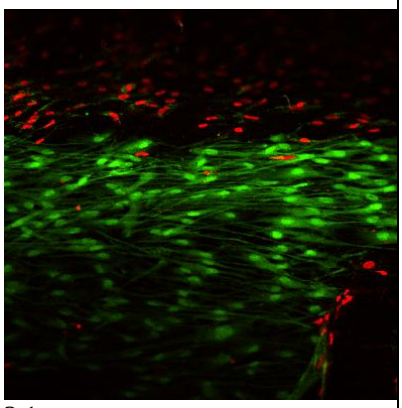
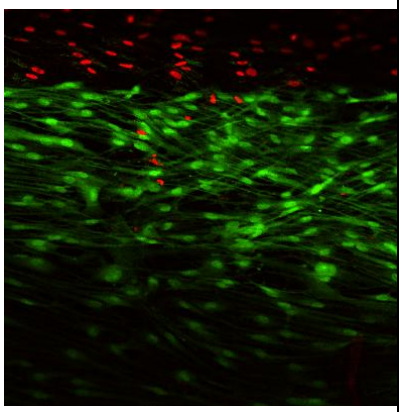
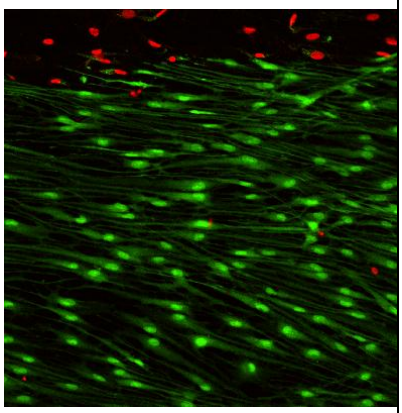
Polylactic acid + ammonia 1	Cells growing on capillary tube. Media remained red	no visible cells	No image
Polylactic acid + ammonia 2	0 mm Media remained red	no visible cells	 2.1
Polylactic acid + ammonia 3	Cells growing on capillary tube + cells growing on tissue culture flask, possibly all the way.	Cells visible all the way along sample	 3.1  3.2

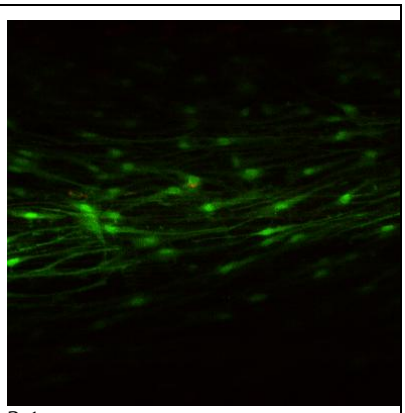
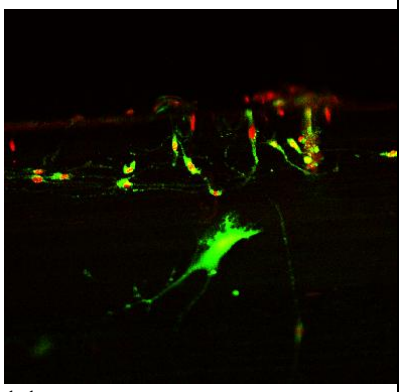
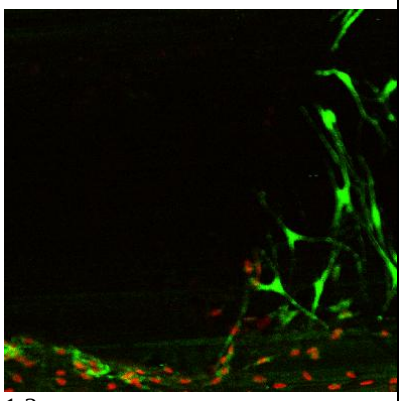
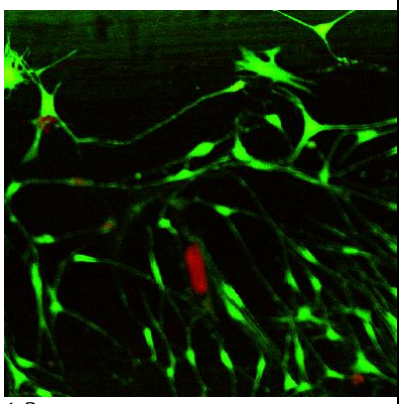
			 3.3  3.4

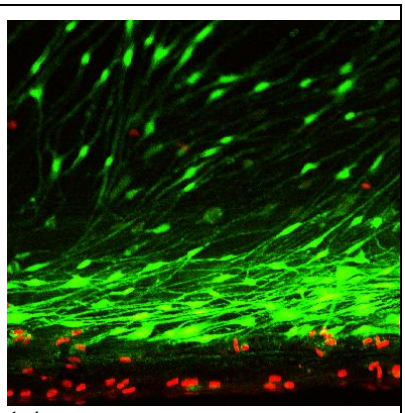
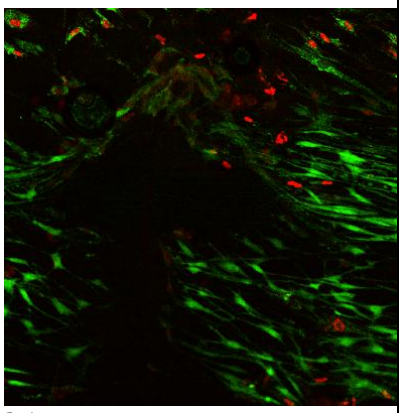
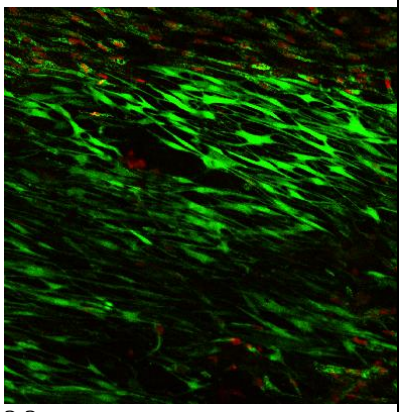
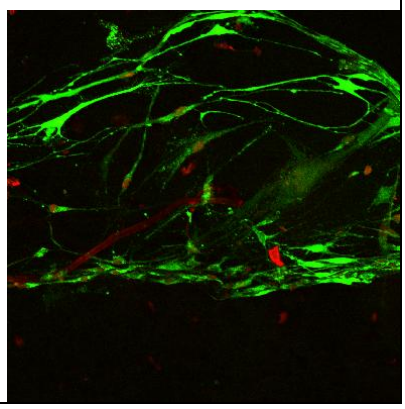
Day	0.0	29.0	31.0	
Date	18/07/2005	16/08/2005	17/08/2005	
Polypropylene 1		0 mm Media remained red	no visible cells	No image
Polypropylene 2		0 mm Media remained red	no visible cells	No image
Polypropylene 3		0 mm Media remained red	no visible cells	No image
Polypropylene + Argon 1		Cells growing on capillary tube + cells growing on tissue culture flask can't measure improvement. Media turned orange.	24.0	 1.1  1.2  1.3

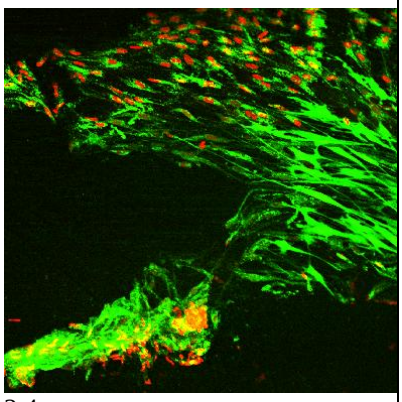
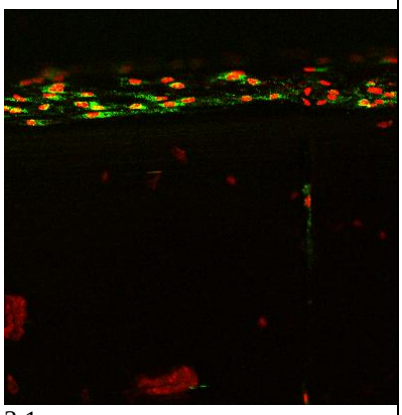
			 1.4  1.5 end
Polypropylene + Argon 2	Cells growing on capillary tube + cells growing on tissue culture flask can't measure improvement. Media turned orange.	24.0mm pic22 at end, pic23 is start end	 2.1  2.2 end

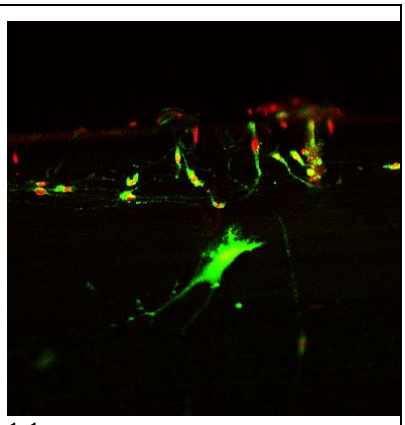
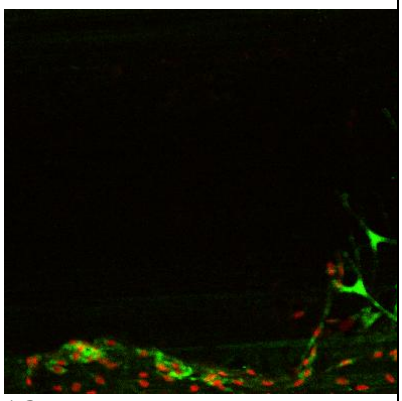
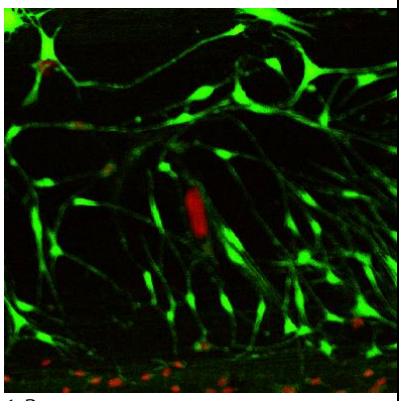
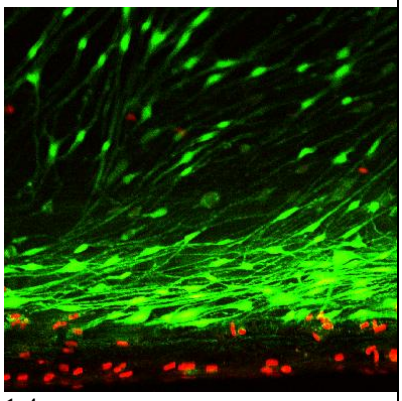
			 2.3  2.4
Polypropylene + Argon 3	0 mm Media remained red	Cells can be seen growing along the edge of the material only.	 3.1
Polypropylene + ammonia 1	Cells growing on capillary tube + cells growing on tissue culture flask can't measure improvement. Media turned orange.	28.0	 1.1

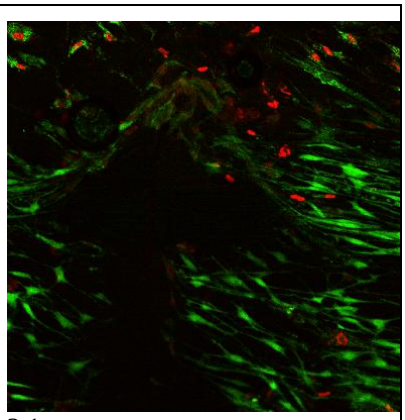
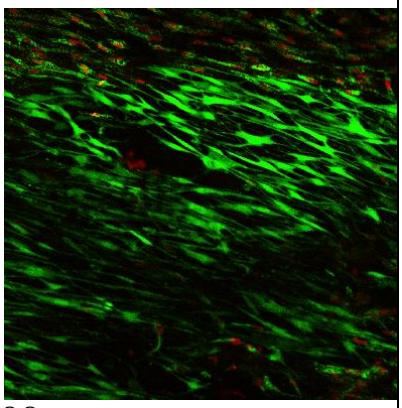
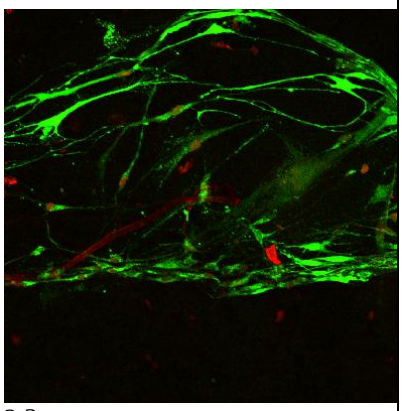
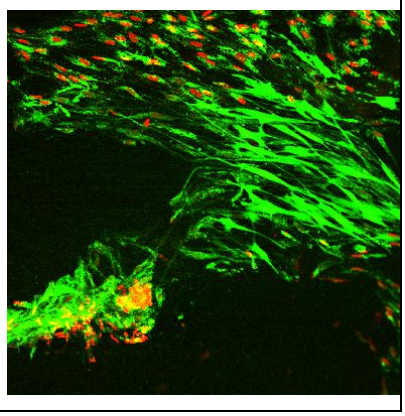
			 1.2
Polypropylene + ammonia 2	Cells growing on capillary tube + cells growing on tissue culture flask can't measure improvement. Media turned orange.	26.0	 2.1  2.2  2.3

Polypropylene + ammonia 3	Cells growing on capillary tube + cells growing on tissue culture flask can't measure improvement. Media turned orange.	20 picture 36	 3.1
Polypropylene + Chitosan 1	0 mm. Piece of detached film floating in media. Media remained red		 1.1  1.2  1.3

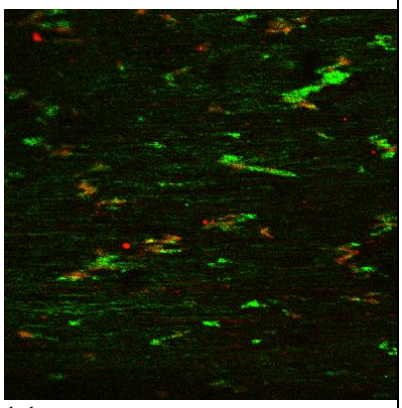
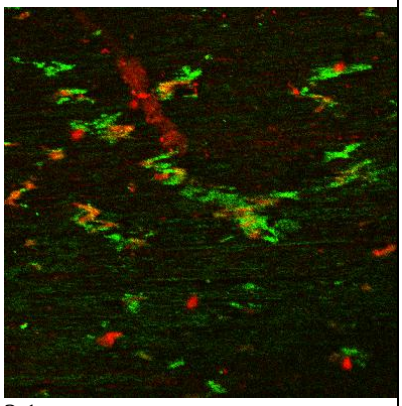
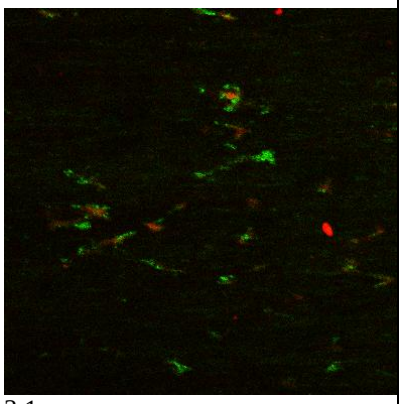
			 1.4
Polypropylene + Chitosan 2	Cells growing on capillary tube + cells growing on tissue culture flask can't measure improvement. Media turned orange.	16.0	 2.1  2.2 

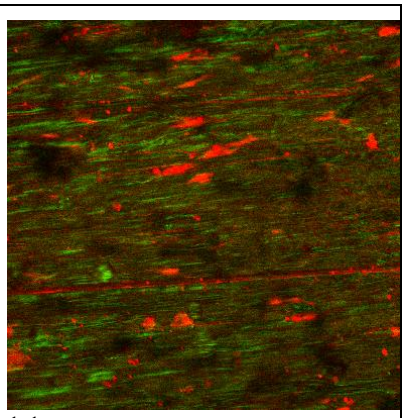
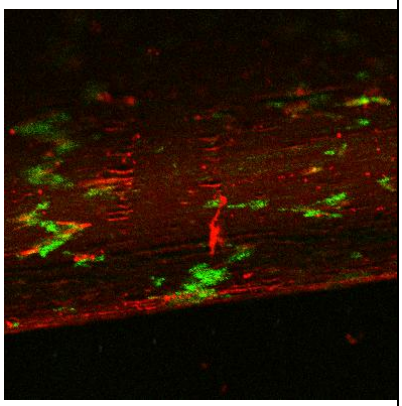
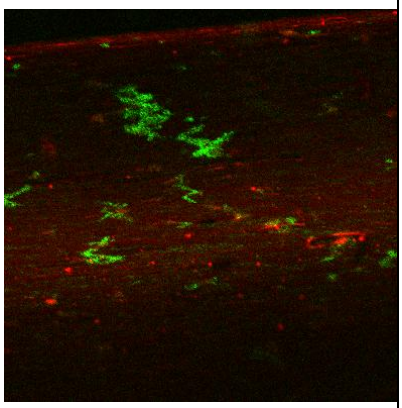
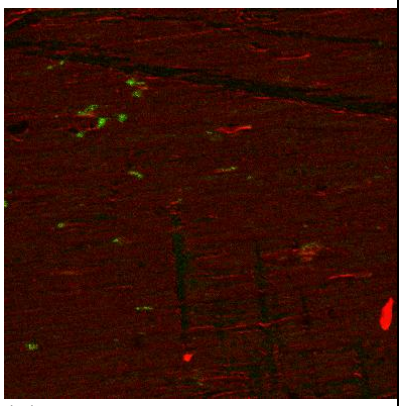
			2.3  2.4
Polypropylene + Chitosan 3	0mm? Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	no visible cells	No image
Polypropylene + Argon + Chitosan 1	0 mm Media remained red	Sample destroyed	No image
Polypropylene + Argon + Chitosan 2	Cells growing on capillary tube + cells growing on tissue culture flask can't measure improvement. Media turned orange.	no visible cells	No image
Polypropylene + Argon + Chitosan 3	1/4 mm? Remaining length looks empty. Media turned orange. cells growing on tissue culture flask	patches along edge	 3.1

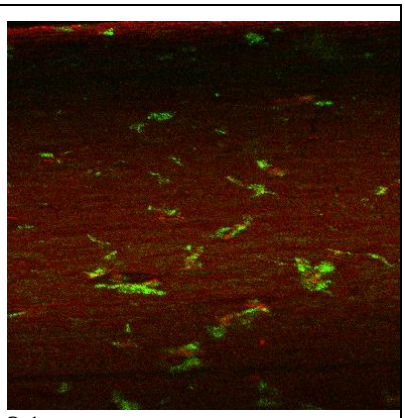
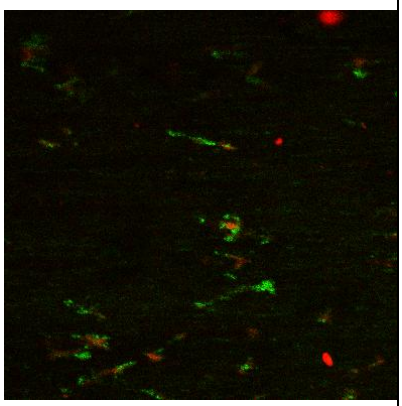
Polypropylene + ammonia + Chitosan 1	Cells growing on capillary tube + cells growing on tissue culture flask can't measure improvement. Media turned orange.	not consistent	 1.1  1.2  1.3  1.4

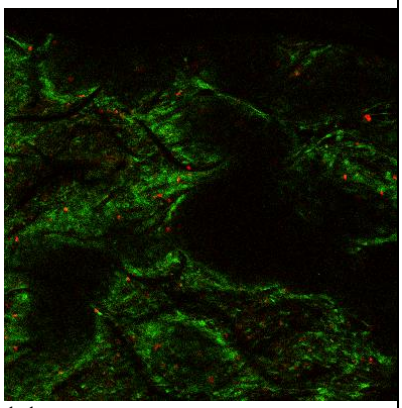
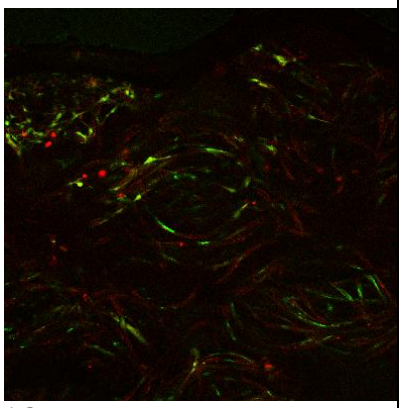
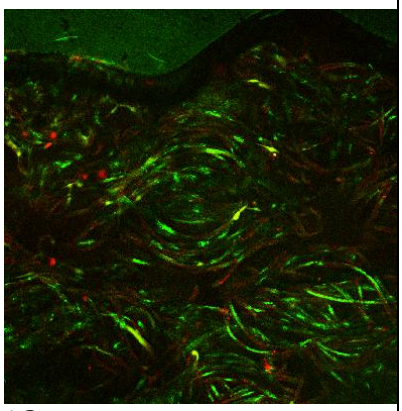
Polypropylene + ammonia + Chitosan 2	Cells growing on capillary tube + cells growing on tissue culture flask can't measure improvement. Media turned orange.		 2.1  2.2  2.3 

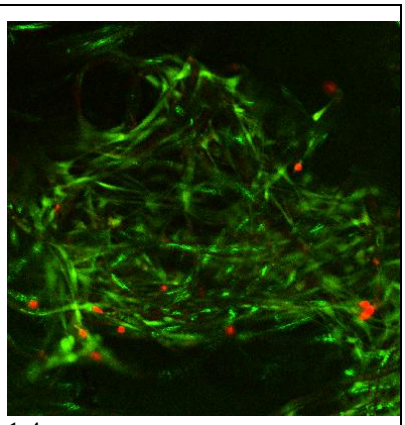
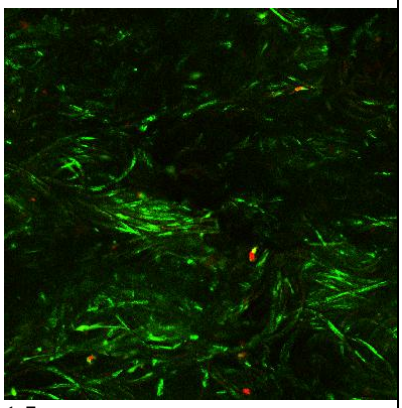
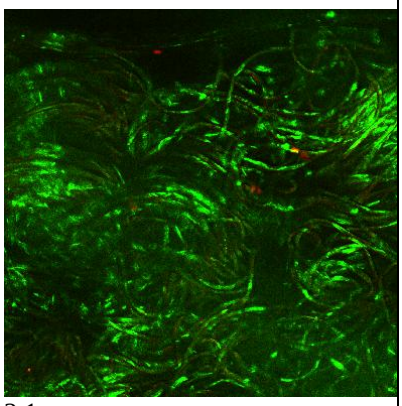
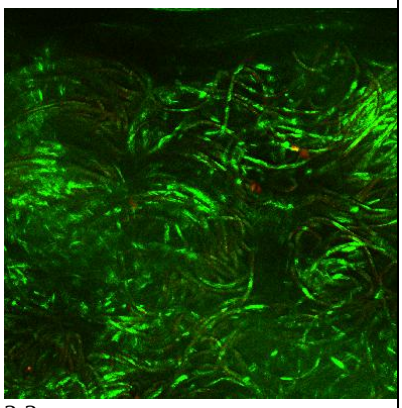
Polypropylene + ammonia + Chitosan 3	0mm. cell debris	no visible cells	No image

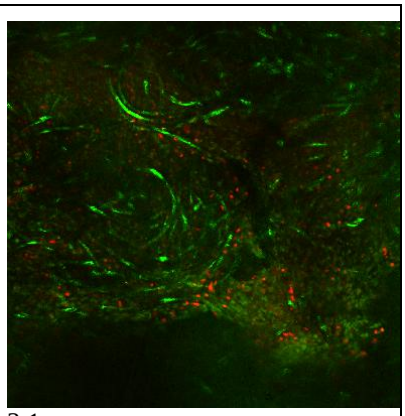
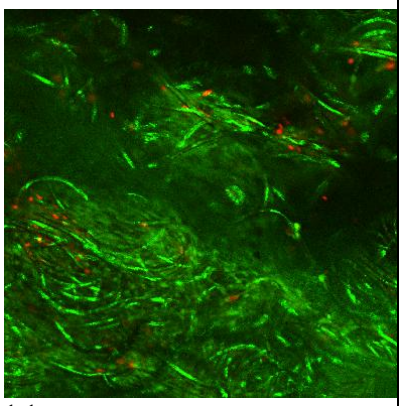
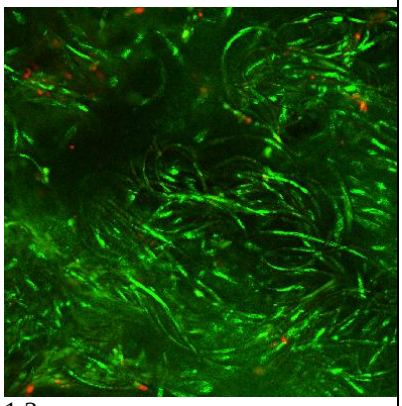
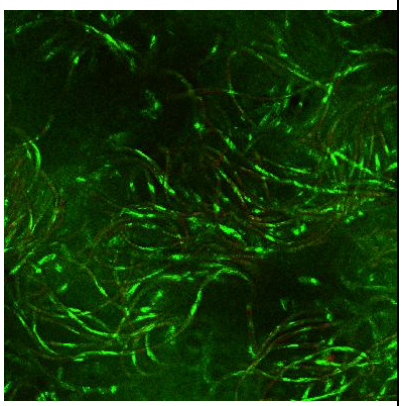
Day	0	29.0	30.0	
Date	18/07/2005	16/08/2005	17/08/2005	
polycaprolactone 1		0 mm Media remained red	Unclear, but unlikely to be cell growth	 1.1
polycaprolactone 2		6 mm plus + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange. cell debris	Unclear, but unlikely to be cell growth	 2.1
polycaprolactone 3		0 mm Media remained red	Unclear, but unlikely to be cell growth	 3.1

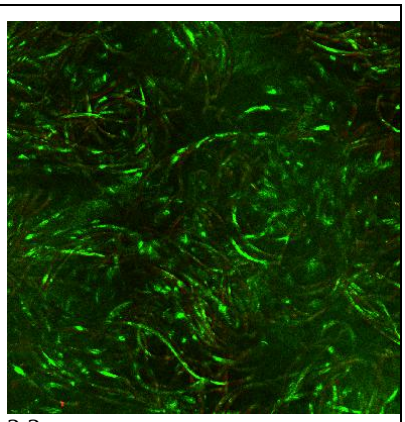
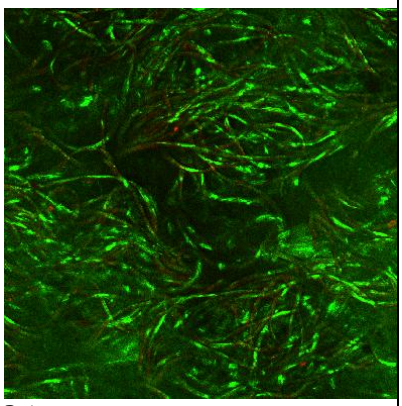
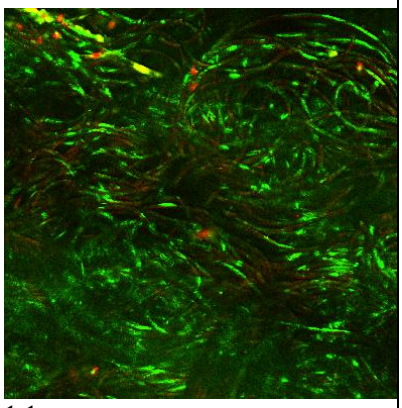
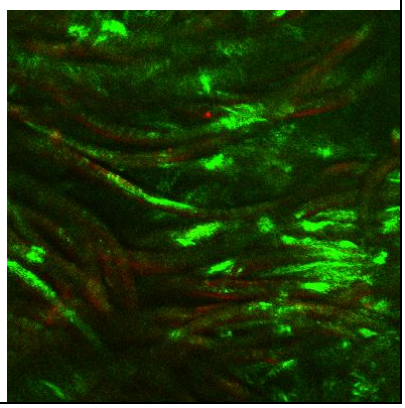
polycaprolactone + Argon 1	0 mm Media remained red	Unclear, but unlikely to be cell growth	 1.1
polycaprolactone + Argon 2	Few cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Unclear, but unlikely to be cell growth	 2.1
polycaprolactone + Argon 3	0 mm Media remained red	Unclear, but unlikely to be cell growth	 3.1
polycaprolactone + ammonia 1	0 mm Media remained red	Unclear, but unlikely to be cell growth	 1.1

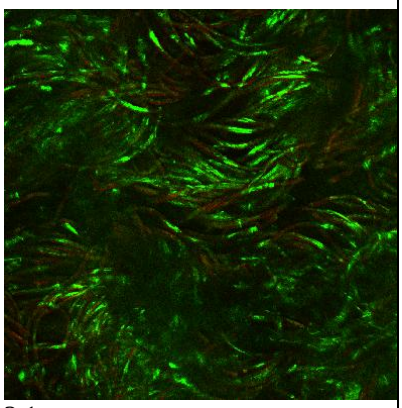
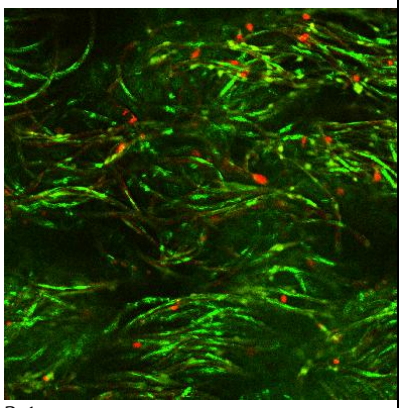
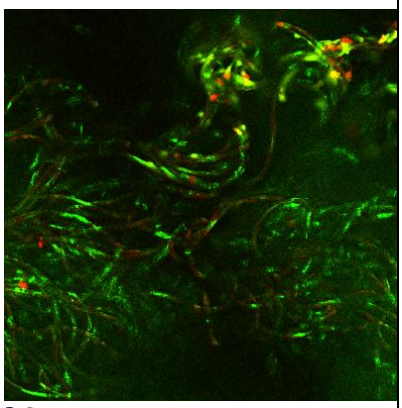
polycaprolactone + ammonia 2	0 mm Media remained red	Unclear, but unlikely to be cell growth	 2.1
polycaprolactone + ammonia 3	0 mm Media remained red	Unclear, but unlikely to be cell growth	 3.1

Day	0	29.0	30.0	
Date	03/08/2005	01/09/2005	02/09/2005	
Polyester 1		7.5 mm (patches of 5, 2 and .5 mm, + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Cells visible all the way along sample? (Error reading?)	 1.1  1.2  1.3

			 1.4 zoom  1.5
Polyester 2	0 mm Media remained red	Cells visible all the way along sample? (Error reading?)	 2.1  2.2

Polyester 3	5 - 8.75 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Cells visible all the way along sample (error reading?)	 3.1
Polyester + Argon 1	4 mm. Media turned orange.	Cells visible all the way along sample? (Error reading?)	 1.1  1.2
Polyester + Argon 2	0 mm Media remained red	Cells visible all the way along sample? (Error reading?)	 2.1

			 2.2
Polyester + Argon 3	0 mm Media remained red	Cells visible all the way along sample? (Error reading?)	 3.1
Polyester + ammonia 1	Few cells growing on tissue culture flask Media remained red	Cells visible all the way along sample? (Error reading?)	 1.1 

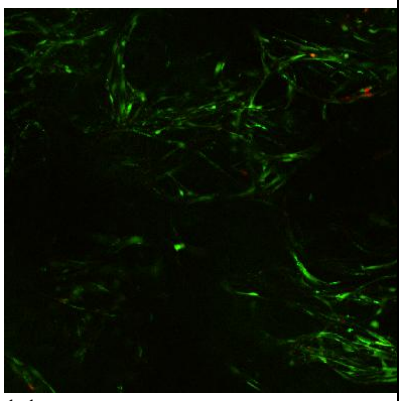
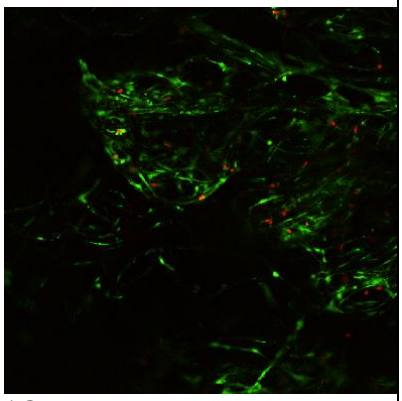
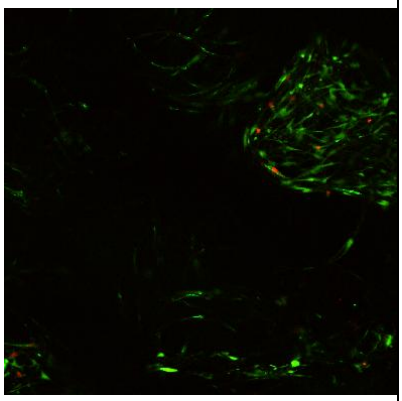
			1.2 zoom
Polyester + ammonia 2	0 mm Media remained red	Cells visible all the way along sample? (Error reading?)	 2.1
Polyester + ammonia 3	7.2 mm (2 one end, 5 other). Media turned orange.	Cells visible all the way along sample? (Error reading?)	 3.1  3.2

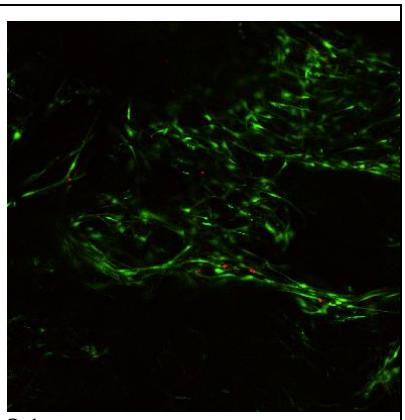
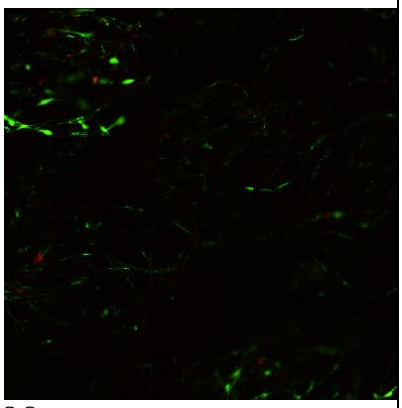
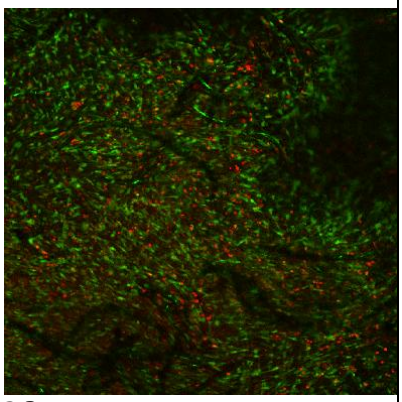
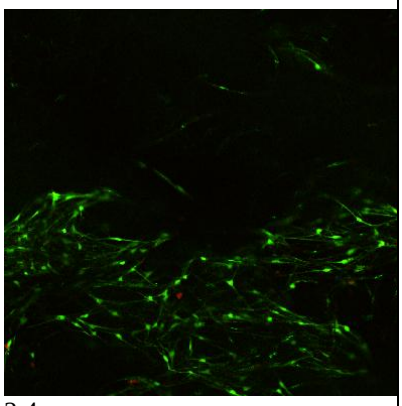
Day	0	29	30	
Date	21/08/2005	19/09/2005	20/09/2005	
Polyurethane 1		0 mm Media remained red	no visible cells	No image
Polyurethane 2		30 mm (dense and loose bits, 2mm d, 4mm l, 6mm d, 8mm l, 4mm d, 2,2mm l) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	no growth beyond 1/3 (2.1 very difficult to visualise) patchy, non-confluent	No image
Polyurethane 3		24 mm (1mm dense, 6mm gap/loose, 23 mm dense or can't see) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	(5 cells, nothing else)	No image
Polyurethane + Argon 1		30 mm (confluent) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Cells visible all the way along sample. Good growth. Confluent, gaps where removed from dish	No image
Polyurethane + Argon 2		0 mm Media remained red	no visible cells	No image
Polyurethane + Argon 3		30 mm (confluent) + cells growing on capillary tube + cells growing on tissue culture flask. Media	Good growth. Continuous along edge both sides. Plenty in	No image

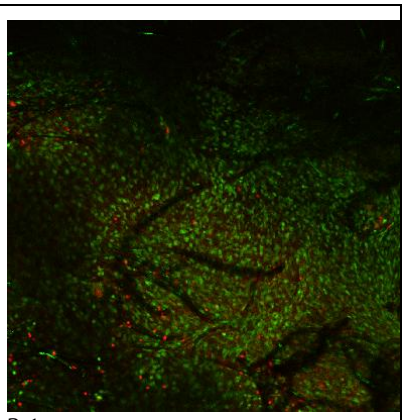
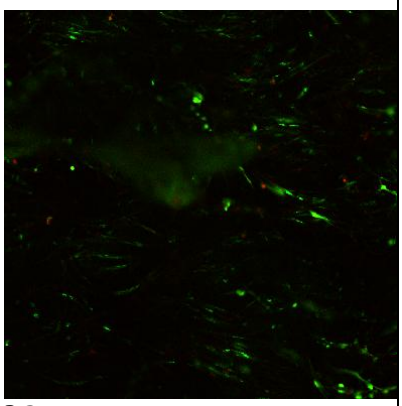
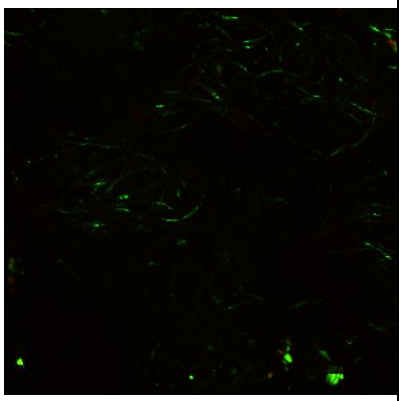
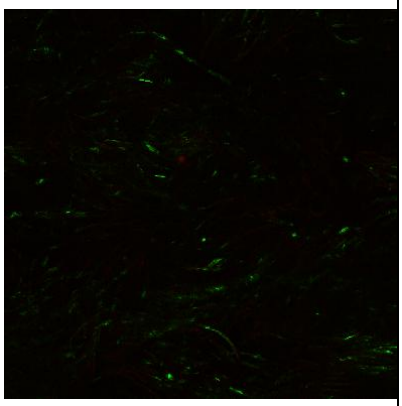
	turned orange.	middle. All the way	
Polyurethane + ammonia 1	30 mm (confluent) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Good growth. Confluent all the way	No image
Polyurethane + ammonia 2	30 mm (confluent) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Gap from 6-12mm. Good growth. Non-confluent. Gaps surrounded by cells	No image
Polyurethane + ammonia 3	30 mm (confluent) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Patchy growth. Mostly along edge. Not dense. All the way	No image
Polyurethane + Argon + Chitosan powder 1	30 mm (confluent) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Very good growth. Confluent all the way, both sides. Excellent	No image
Polyurethane + Argon + Chitosan powder 2	0 mm Media remained red	no visible cells	No image
Polyurethane + Argon + Chitosan powder 3	0 mm (signs of dead cells) Media remained red	no visible cells	No image
Polyurethane + Argon @ Galashiels 1	30 mm (confluent) + cells growing on capillary tube + cells	Cells confluent all the way along sample.	No image

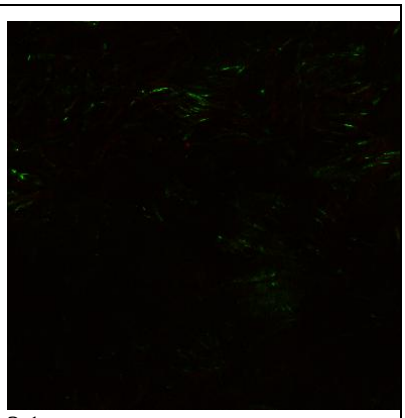
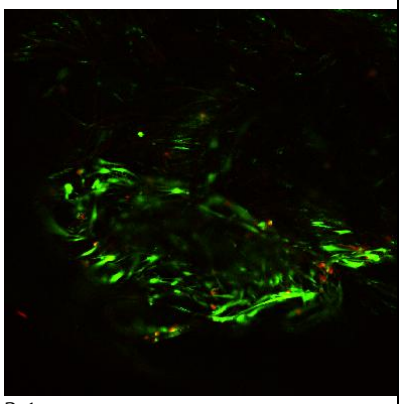
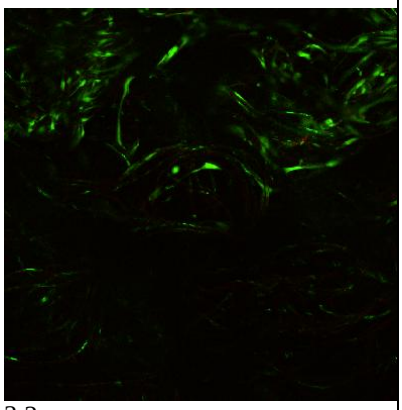
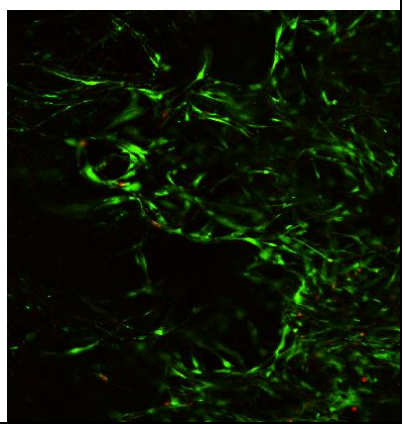
	growing on tissue culture flask. Media turned orange.	Dense	
Polyurethane + Argon @ Galashiels 2	6mm v. loose, v. little clump of cells growing on tissue culture flask, no cells growing on capillary tube. Media turned orange.	no visible cells	No image
Polyurethane + Argon @ Galashiels 3	0 mm signs of dead cells at one end Media remained red	no visible cells	No image
Polyurethane + Argon @ Galashiels 4	0 mm Media remained red	no visible cells	No image
Polyurethane + Argon @ Galashiels 5	30 mm (confluent) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	Cells confluent all the way along sample. Very dense all the way	No image
Polyurethane + Argon @ Galashiels 6	0 mm Media remained red	no visible cells	No image
Polyurethane + Oxygen @ Galashiels 1	0 mm Media remained red	no visible cells	No image
Polyurethane + Oxygen @ Galashiels 2	0 mm Media remained red	no visible cells	No image
Polyurethane + Oxygen @ Galashiels 3	0 mm Media remained red	no visible cells	No image
Polyurethane + Oxygen @ Galashiels	30 mm (confluent) + cells growing on	Dense for first 5mm. Cells	No image

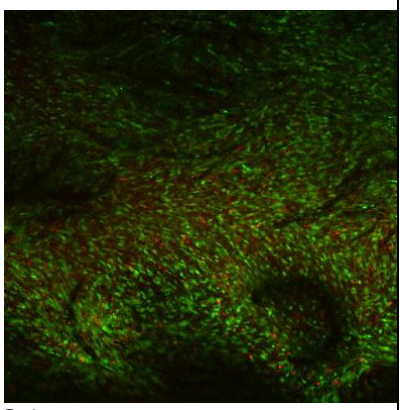
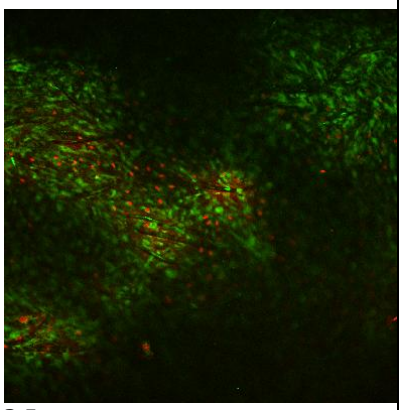
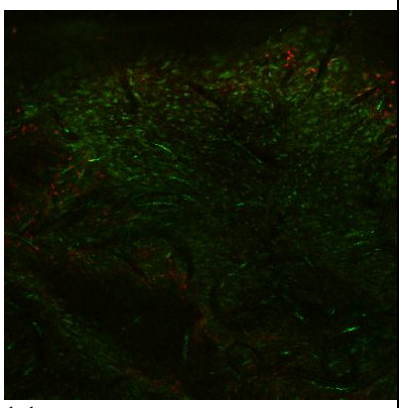
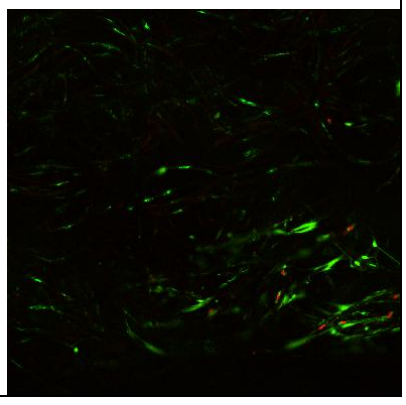
4	capillary tube + cells growing on tissue culture flask. Media turned orange.	thick to 12mm. Not very dense to 28mm. Confluent for last 2mm. Cells probably ripped off in places	
Polyurethane + Oxygen @ Galashiels 5	30 mm (confluent) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	cells confluent on other side (outward facing side, very dense	No image
Polyurethane + Oxygen @ Galashiels 6	0 mm Media remained red	no visible cells	No image

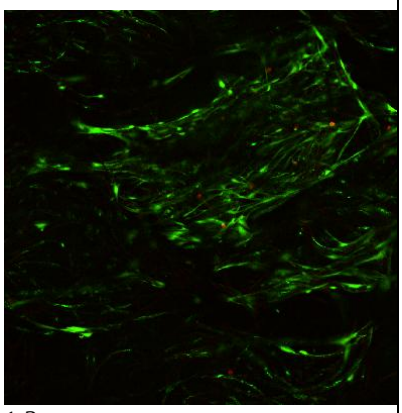
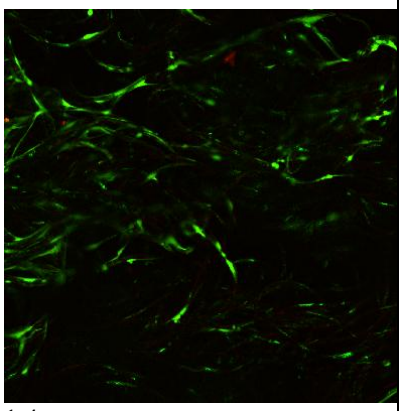
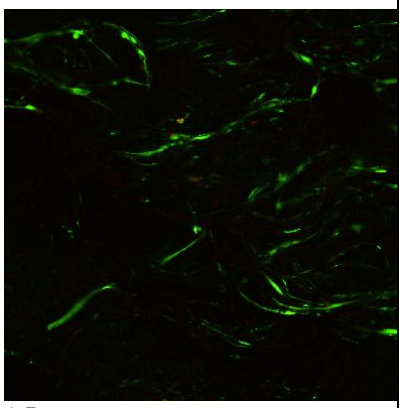
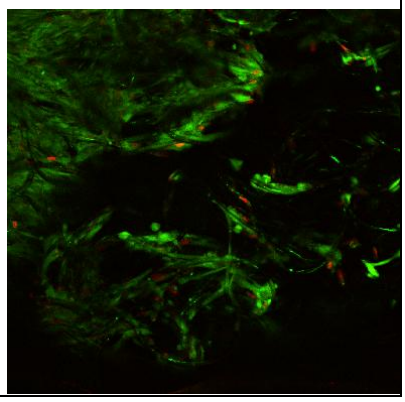
Day	0	29	30	
Date	21/08/2005	19/09/2005	20/09/2005	
Polyester 1		30 mm (dense for first 6mm, cells everywhere + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	1.2 is one end. Other end damaged by removal. 1/4 from other end is picture 1.3. all the way	 1.1  1.2  1.3

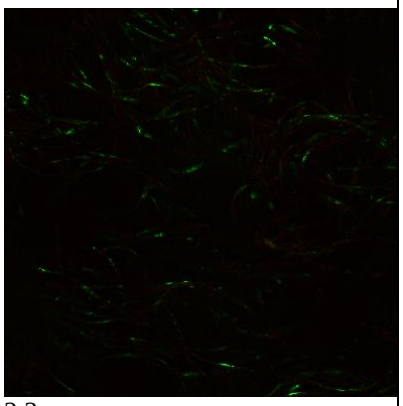
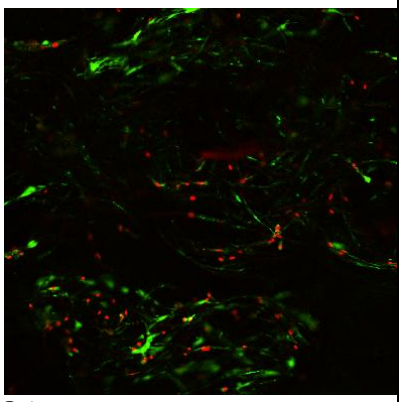
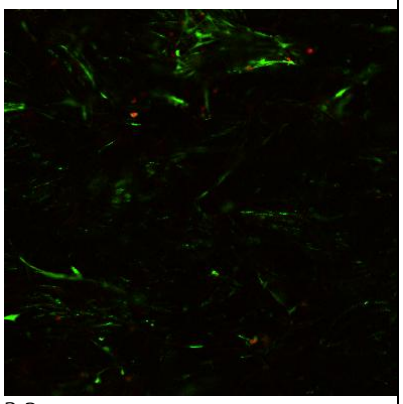
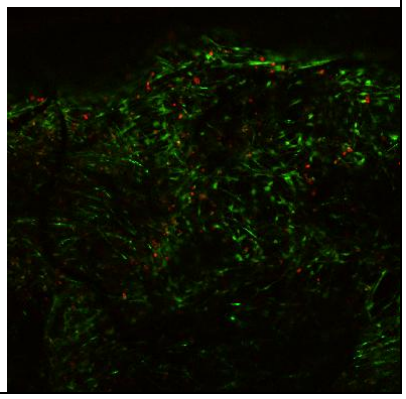
Polyester 2	26 mm (4mm gap 16mm from one end, 10mm from other. 5mm very dense + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	2.2 is one end. Vas 2.3 is the other end. Very dense for about 6mm. 2.4 is past dense bit	 2.1  2.2  2.3  2.4

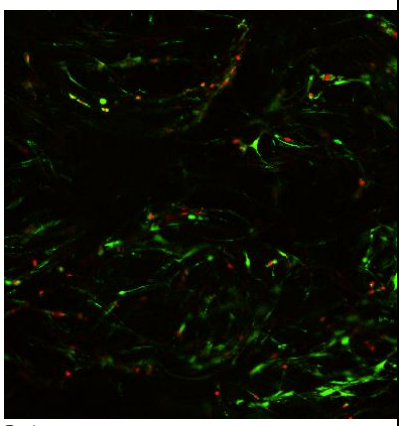
Polyester 3	7 mm (5.5mm dense, 0.5mm less dense, 1mm other end. Rest mostly gap with one or two cells + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	3.1 is very dense section. 3.2 is ~ 6mm from end. Like 3.3 for rest. Little clumping 8mm from 3.1	 3.1  3.2  3.3
Polyester + Argon 1	0 mm traces of dead cells, Media remained red	no visible cells	 1.1

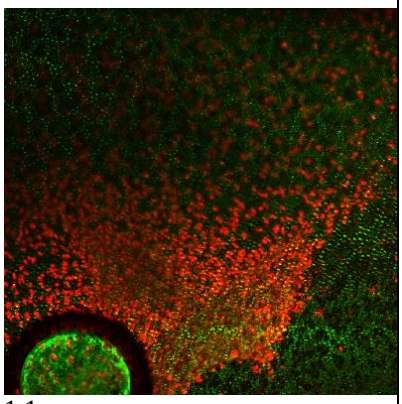
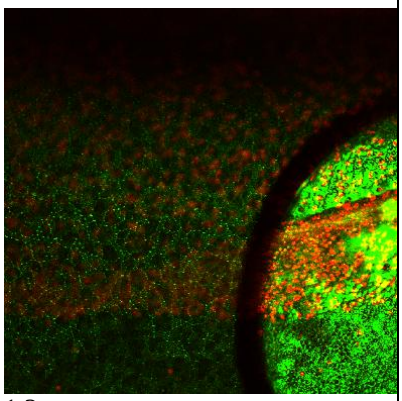
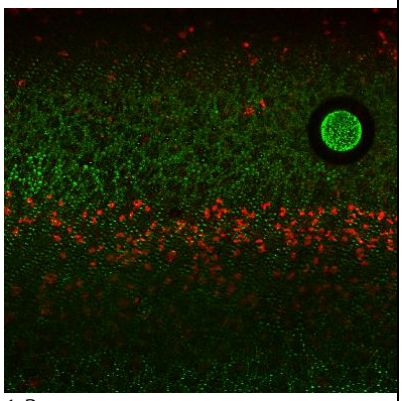
Polyester + Argon 2	0 mm traces of dead cells, Media remained red	no visible cells	 2.1
Polyester + Argon 3	8 mm (7.25mm one end, 0.75mm the other end) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange.	3.1 is one end, gap for~10mm, picture 3.2, loose until 10mm from end (picture 3.3) then confluent and dense until end.	 3.1  3.2 

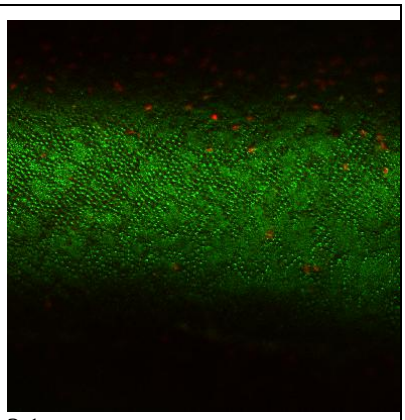
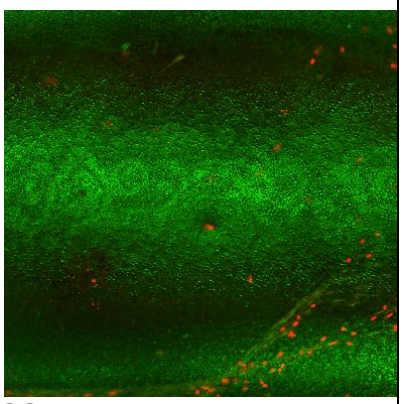
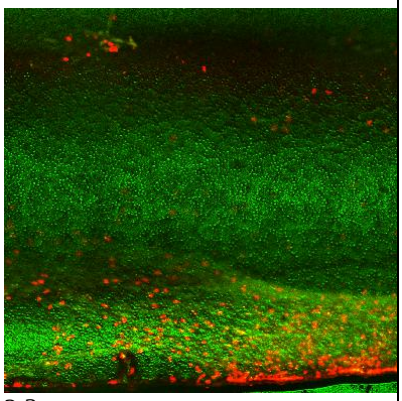
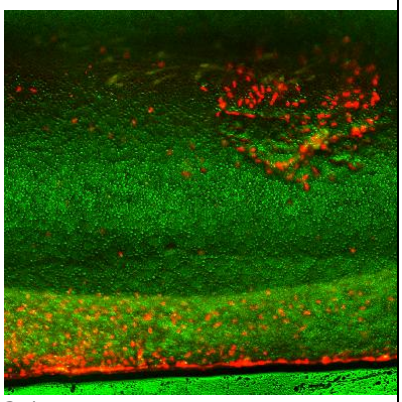
			3.3  3.4  3.5
Polyester + ammonia 1	9mm (7mm one end, dense, other end, 1.25mm, 5.75mm gap then 0.75mm) + cells growing on tissue culture flask. Media turned orange.	1.1 is one end. All the way, but mostly not very dense, like 1.4 &1.5. 1.3 is mid, 1.4 and 1.5 is either side	 1.1 

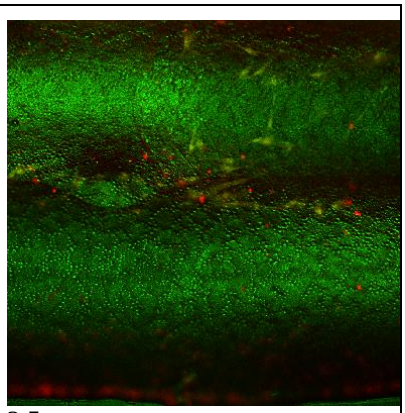
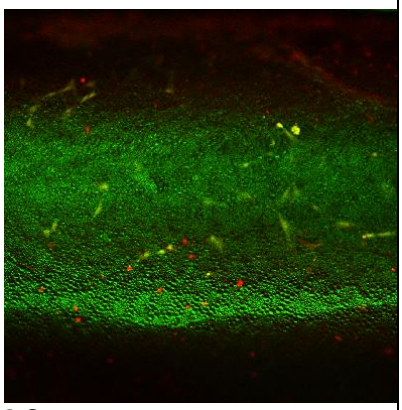
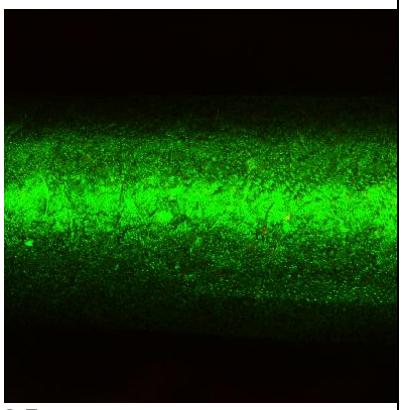
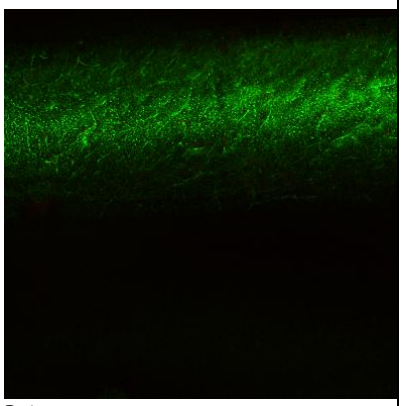
			1.2  1.3  1.4  1.5
Polyester + ammonia 2	7 mm. Media turned orange	2.1 is 10mm from dense end, then nothing (like picture 2.2) to other end.	

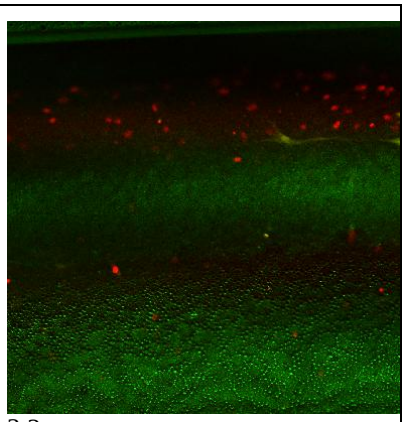
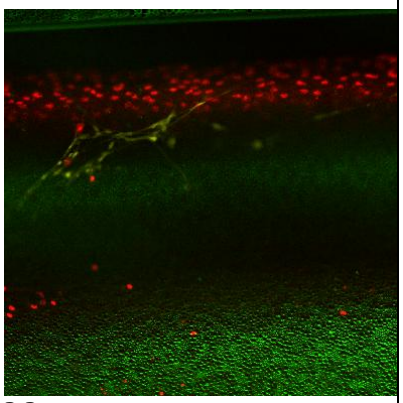
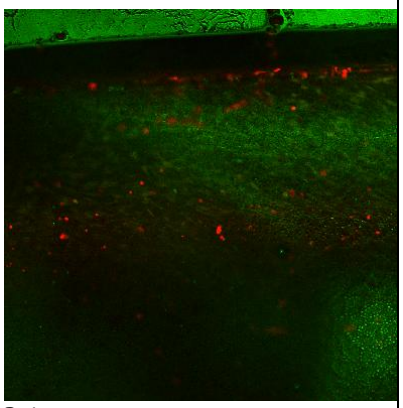
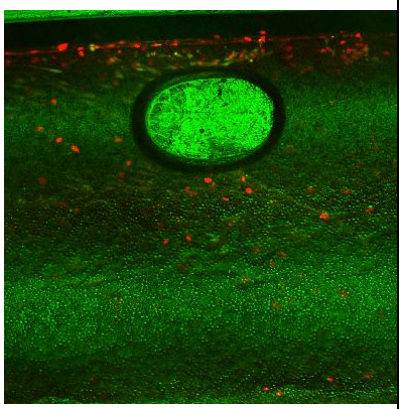
			2.1  2.2
Polyester + ammonia 3	30 mm (9mm v. dense, dense all the way) + cells growing on capillary tube + cells growing on tissue culture flask	3.1 is one end, like that for 5mm, then less dense like 3.2 for 2mm, then more dense like 3.3 for last 5mm	 3.1  3.2 

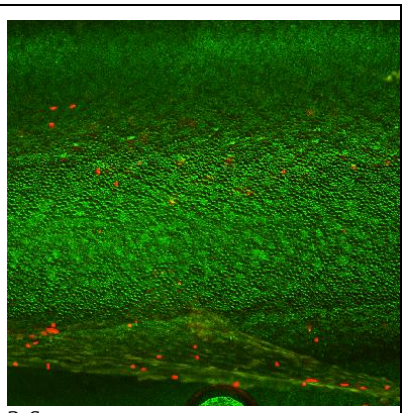
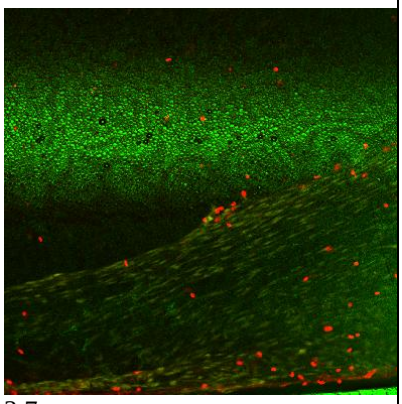
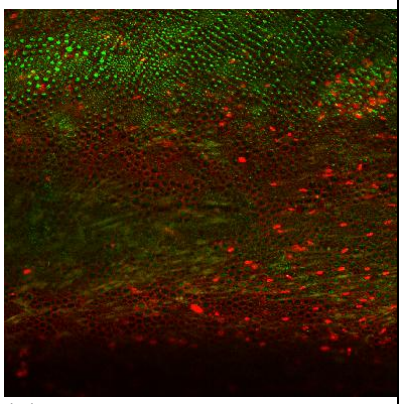
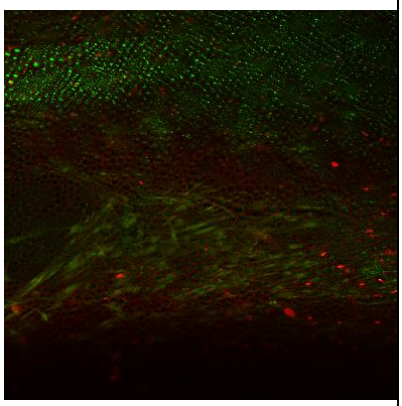
			3.3  3.4

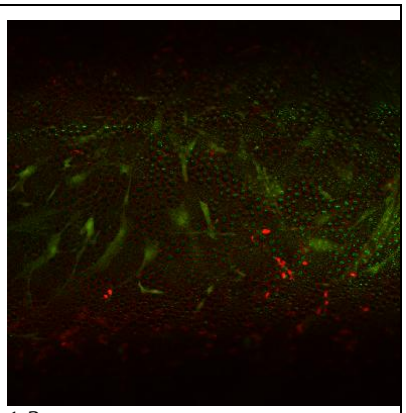
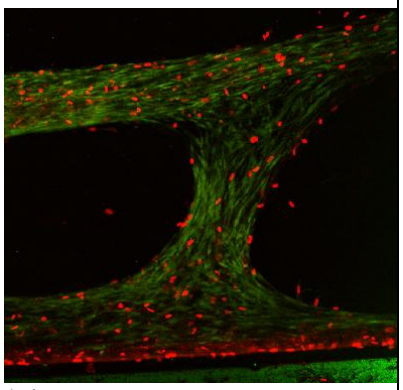
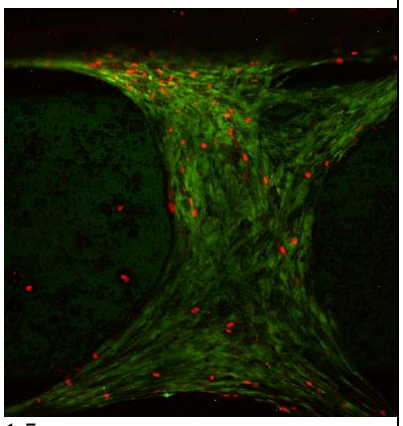
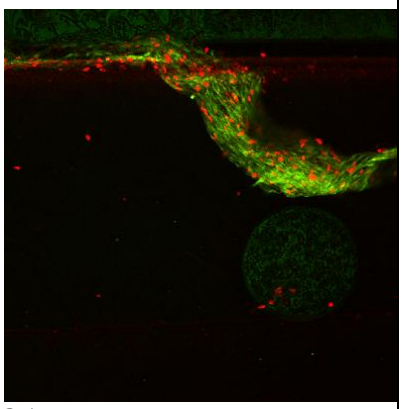
Day	0	29	30	
Date	21/08/2005	19/09/2005	20/09/2005	
Polylactic acid 1		Cell attachment at both ends. Can't measure, probably all the way. Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	fluorescence indicates nothing resembling live cells present	 1.1  1.2  1.3

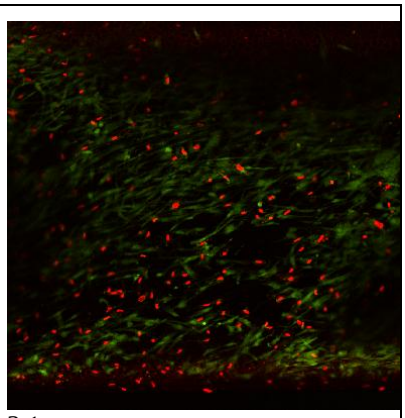
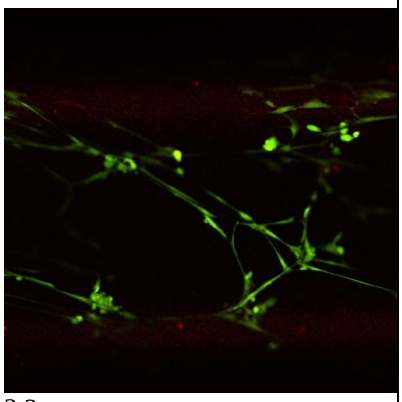
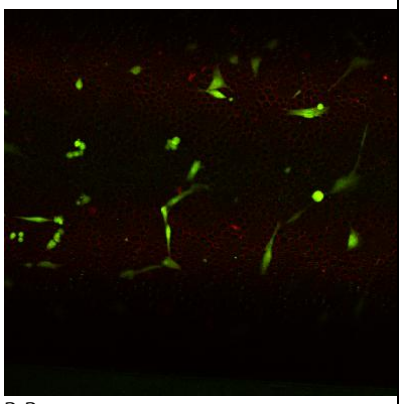
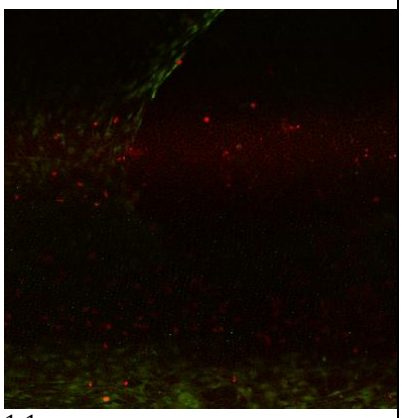
Polylactic acid 2	Cell attachment at both ends. Can't measure, probably all the way. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	if this is cell growth, then its all the way , patchy in places(see picture)	 2.1  2.2  2.3  2.4

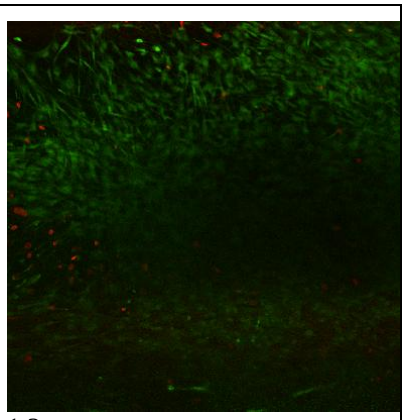
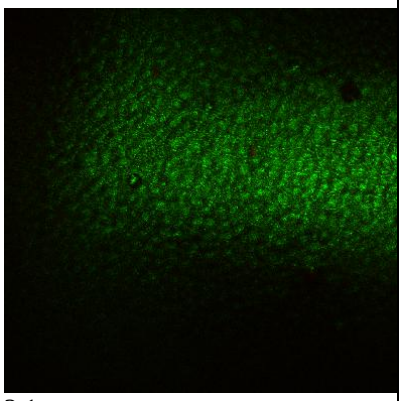
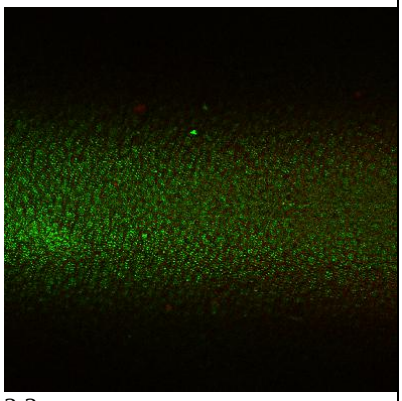
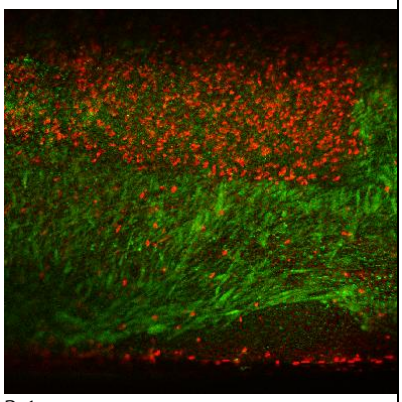
			 2.5  2.6  2.7
Polylactic acid 3	Cell attachment at both ends. Can't measure, probably all the way. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Picture 3.2 = one end, 3.4 = the other, picture 3.7 = dense bit. Cells visible all the way	 3.1

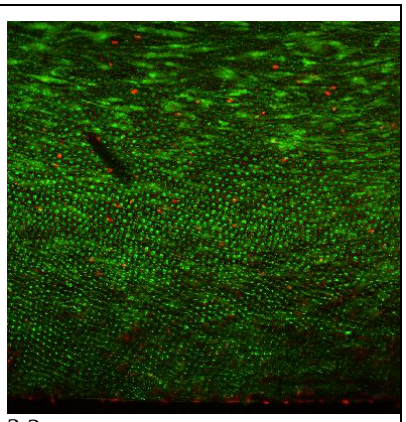
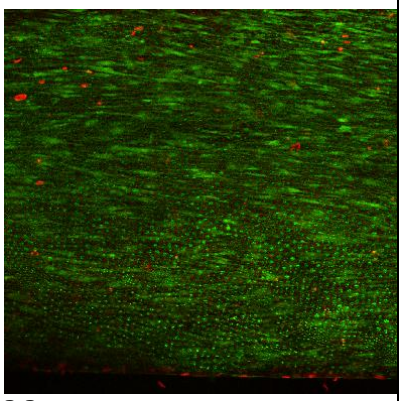
			 3.2  3.3  3.4  3.5

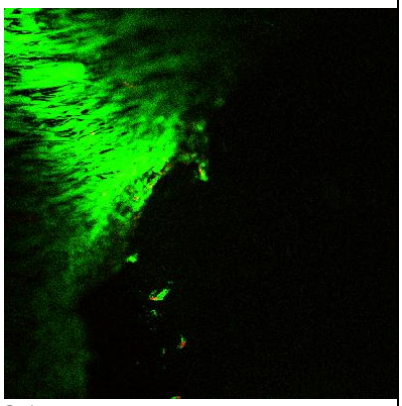
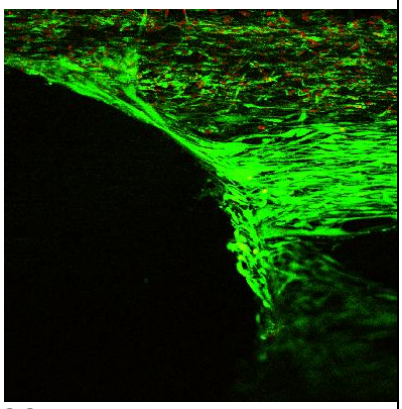
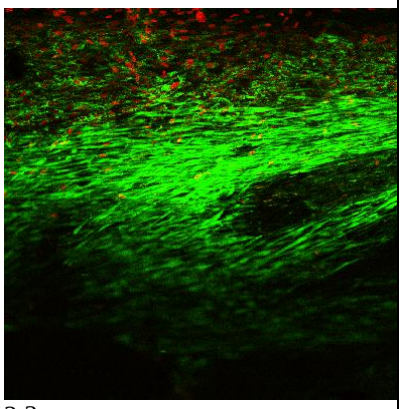
			 3.6  3.7
Polylactic acid + Argon 1	Cell attachment at both ends. Can't measure, probably all the way. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	All the way, mostly on edge (avoiding artefacts) picture 1.4 and picture 1.5. spanning width occasionally	 1.1  1.2

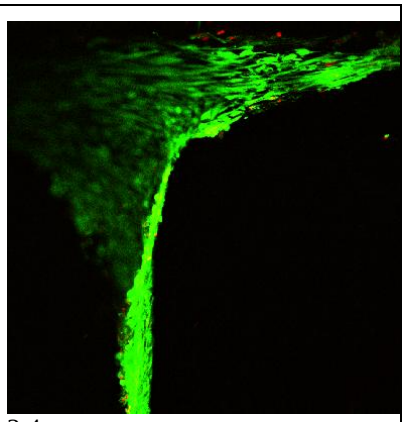
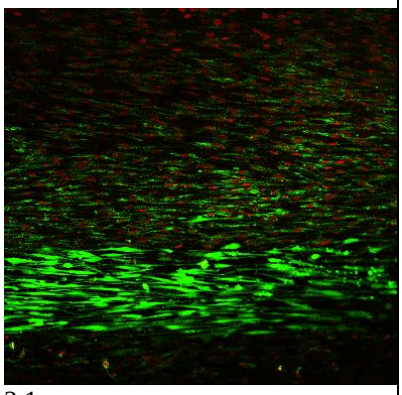
			 1.3  1.4  1.5
Polylactic acid + Argon 2	0 mm Media remained red	1st picture is cells between 14-18mm (approximately) that is all	 2.1

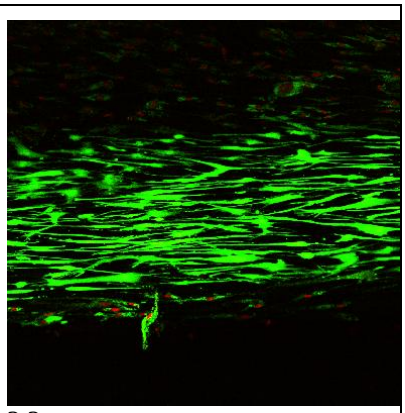
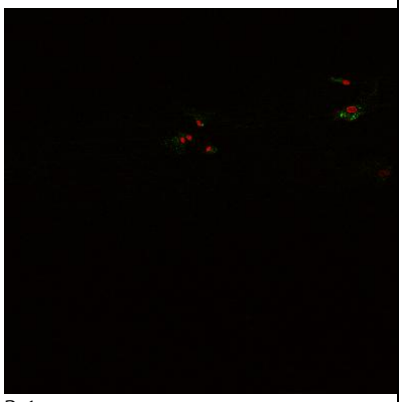
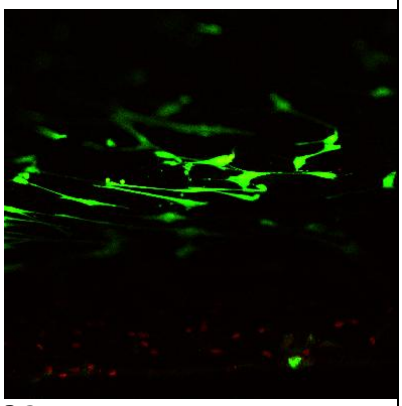
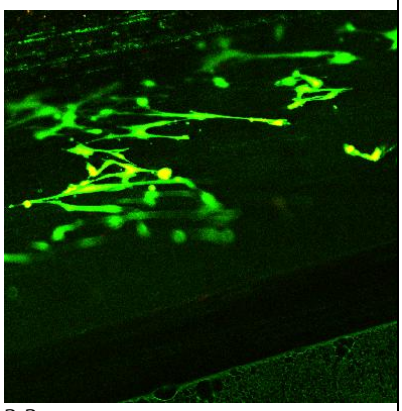
Polylactic acid + Argon 3	Cell attachment at one end, can't measure + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Picture 33 is typical density. Picture 3.2 is 1/4 way up. Cells all the way, but not very dense. One or two gaps on the other end. Probably ripped off from removal from the glass	 3.1  3.2  3.3
Polylactic acid + ammonia 1	Cell attachment at one end, possibly other, can't measure + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Cells grew all the way, but have been lost due mechanical removal of sample from capillary tube.	 1.1

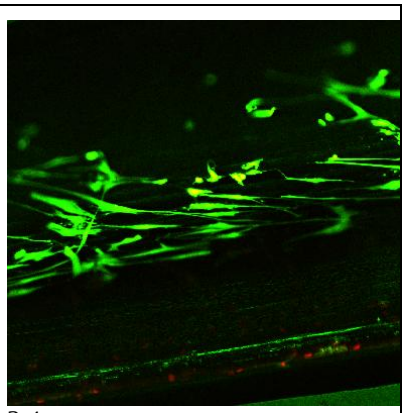
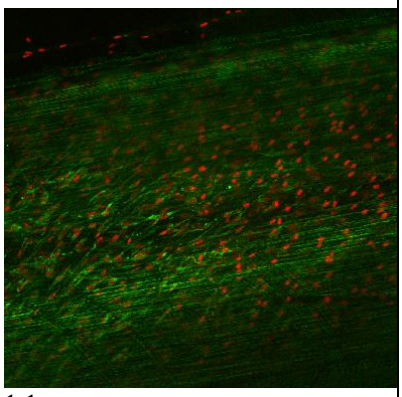
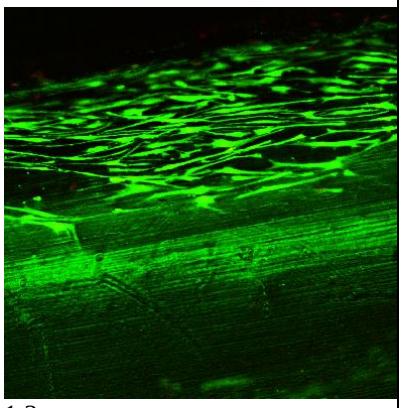
			 1.2
Polylactic acid + ammonia 2	0 mm Media remained red	no visible cells	 2.1  2.2
Polylactic acid + ammonia 3	Cell attachment at one end, possibly other, can't measure + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Picture 3.1 is one end, confluent, same all the way.	 3.1

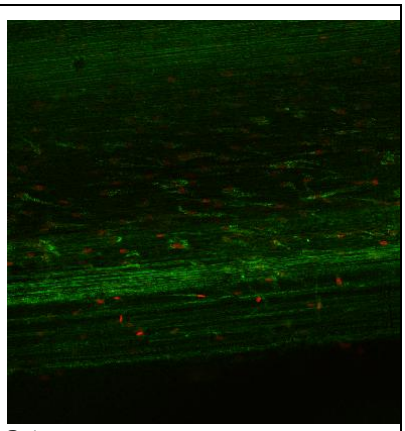
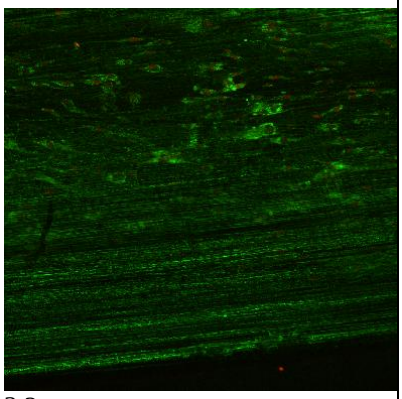
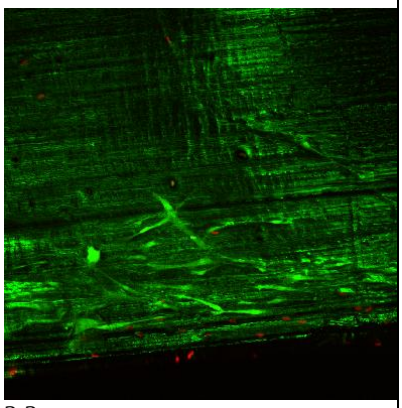
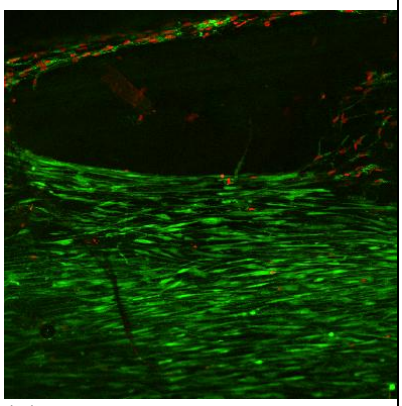
			 3.2  3.3

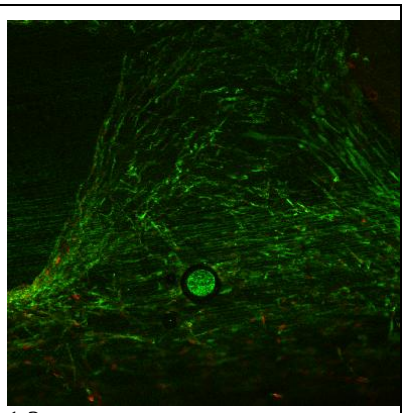
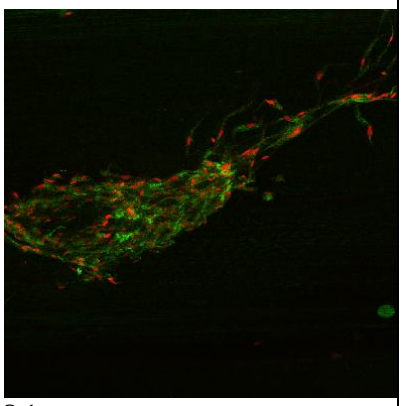
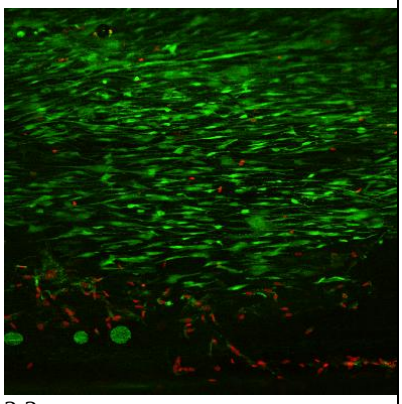
Day	0	29	30	
Date	25/08/2005	23/09/2005	24/09/2005	
Polypropylene 1		0 or 6 mm (possibly 6mm patch 6mm from one end, or no cells.) Media remained red	no visible cells	No image
Polypropylene 2		Cells attached and growth along sample. Can't see clearly enough to measure. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange /red	There were more cells. Appears a lot have been ripped off. Picture 2.2 is ripped site. 5mm from one end; 12mm is empty/ ripped off. Cells remain on 10mm of other end.	 2.1  2.2  2.3

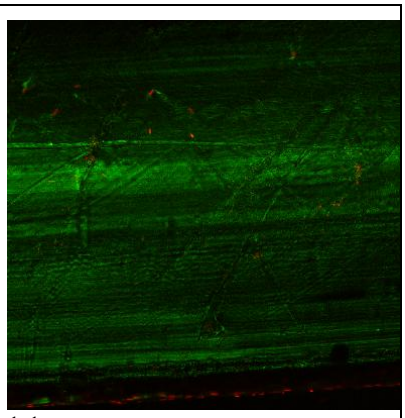
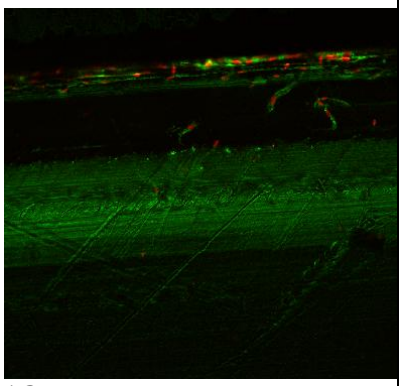
			 2.4
Polypropylene 3	Cells attached and growth up. Can't see clearly enough to measure. Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Looks like what cells were there are dead by now. Similar appearance to pp2. No live cells left.	No image
Polypropylene + Argon 1	0 mm? Media remained red	no visible cells	No image
Polypropylene + Argon 2	Definite attachment one end. Can't see well enough to measure. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Confluent. Some cells still alive. 2.1 are live and dying cells, 2.2 are also live and dying cells.	 2.1

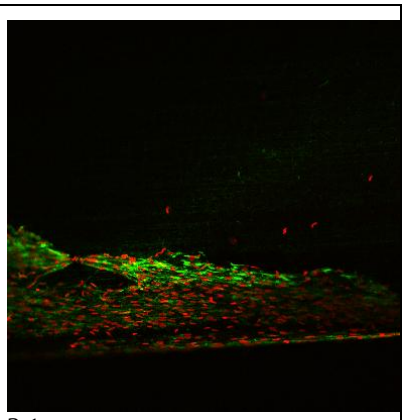
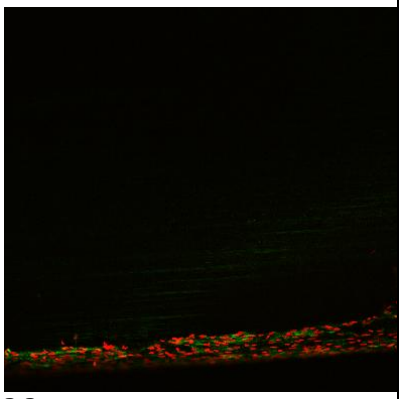
			 2.2
Polypropylene + Argon 3	Can't see attachment. Can tell tomorrow. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Confluent and not very populated bits. 3.1 is empty bit. 3.2 is better bit, 3.3 is as clear as it gets I think. Reasonable. 3.3 for 7mm, virtually empty for 13mm, like 34 for next 7mm, last 3mm pretty empty. Probably mechanical damage from removal of sample from capillary tube.	 3.1  3.2  3.3

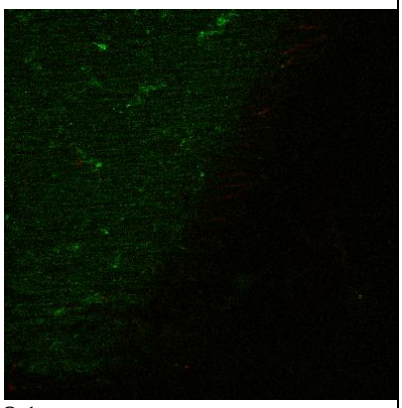
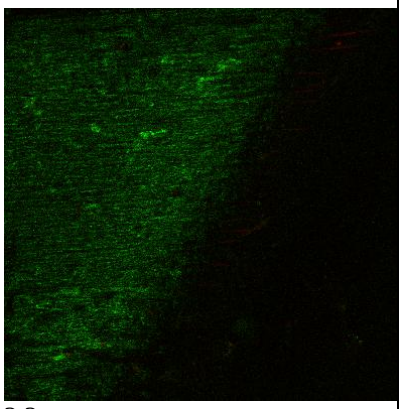
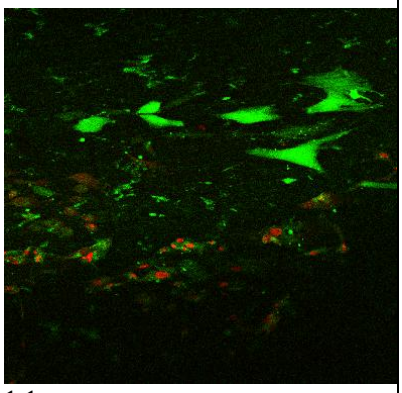
			 3.4
Polypropylene + ammonia 1	Definite attachment one end. Can't see well enough to measure. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Confluent. Cells start dying due to length of time required to examine all of the samples. Picture 1.1 shows some cells alive on the other side with a gap from removal from the capillary tube.	 1.1  1.2
Polypropylene + ammonia 2	0 mm Media remained red	no visible cells	No image

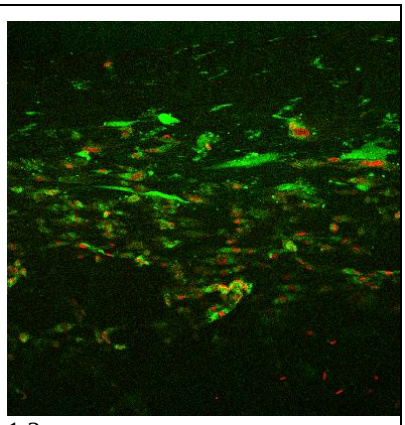
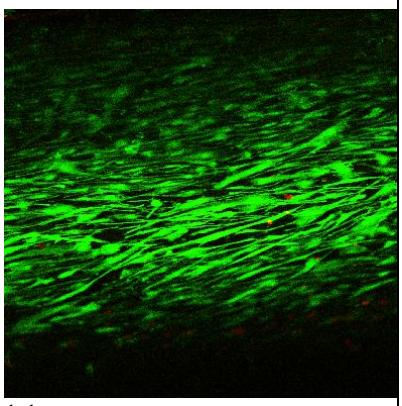
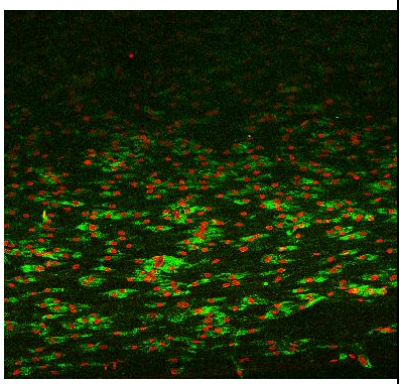
Polypropylene + ammonia 3	Definite attachment both ends. Can't see well enough to measure. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	3.1 and 3.2 unclear (external side). Confluent but cells dying on external side. Cells still alive on inner side.	 3.1  3.2  3.3
Polypropylene + chitosan 1	Definite attachment both ends. Can't see well enough to measure. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Confluent but for gaps where removal from capillary tube caused holes. 1.1 and 1.2	 1.1

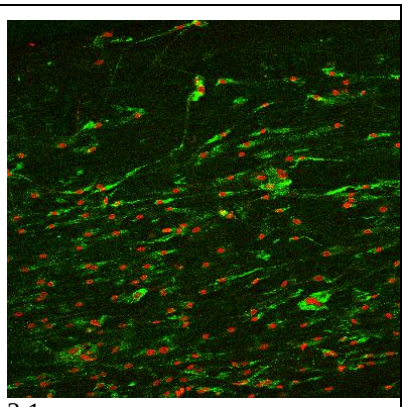
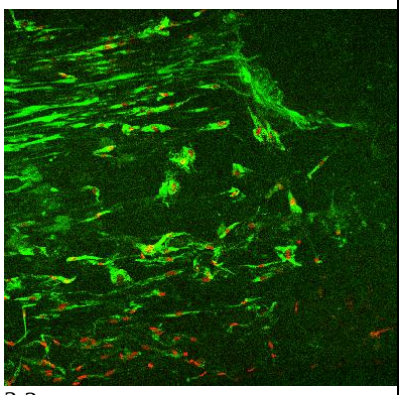
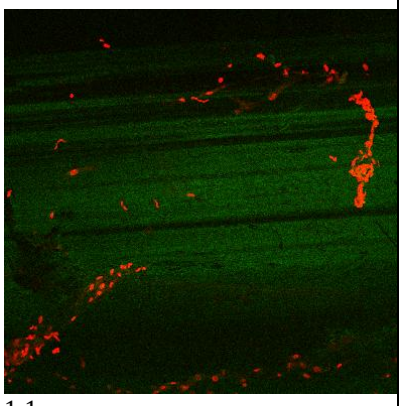
			 1.2
Polypropylene + Chitosan 2	Definite attachment both ends. Can't see well enough to measure. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Confluent in 3 bits. Looks like rest has been ripped off. 2.1 and 2.2 are ripped bits.	 2.1  2.2
Polypropylene + Chitosan 3	0 mm Media remained red	no visible cells	No image

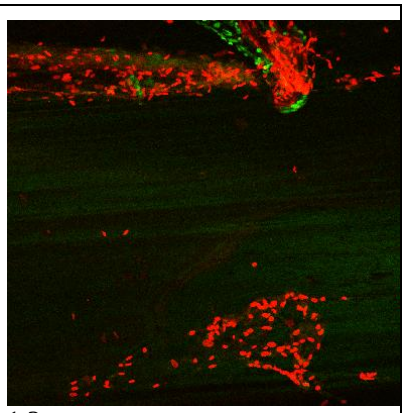
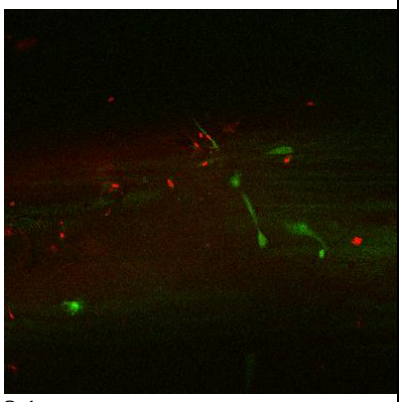
Polypropylene + Argon + Chitosan 1	Definite attachment both ends. Can't see well enough to measure. Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange /red	0 (practically) like 1.1 and 1.2 for all. V. poor)	 1.1  1.2
Polypropylene + Argon + Chitosan 2	0 mm (peeling chitosan film. Think, almost def, none Media remained red	no visible cells	No image
Polypropylene + Argon + Chitosan 3	0 mm. Media remained red	no visible cells	No image
Polypropylene + ammonia + Chitosan 1	0 mm. Media remained red	no visible cells	No image
Polypropylene + ammonia + Chitosan 2	0 mm. Media remained red	no visible cells	No image

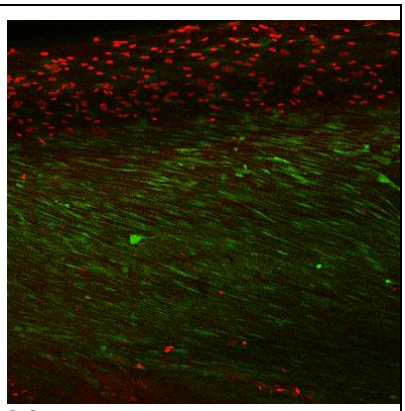
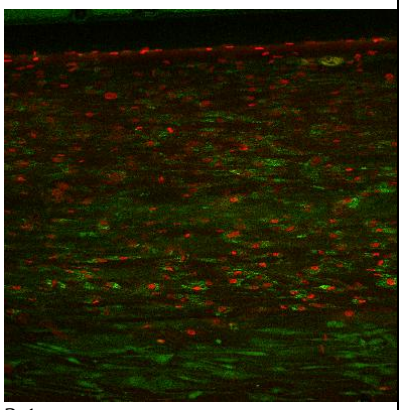
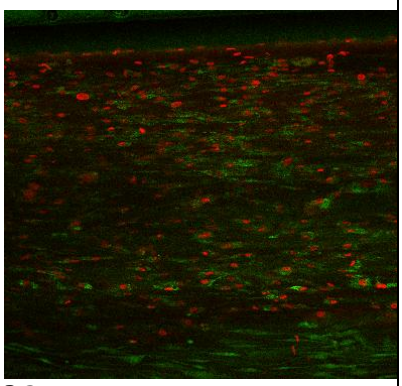
Polypropylene + ammonia + Chitosan 3	Definite attachment both ends. Can't see well enough to measure. cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	just along edge (like bottom right of 3.1 and 3.2 except for 2mm (3.1)	 3.1  3.3

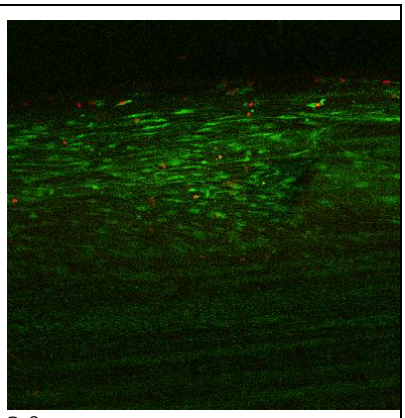
Day	0	29	30	
Date	25/08/2005	23/09/2005	24/09/2005	
Polycaprolactone 1		0. Very few cells growing on capillary tube (~12 cells max). Media remained red	no visible cells	No image
Polycaprolactone 2		Possible attachment. Measure next day. Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange /red	Very few cells.	 2.1  2.2
polycaprolactone 3		0 mm. Media remained red	no visible cells	No image
polycaprolactone + Argon 1		8 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	10mm not very dense. 1.1 is end of cells, 1.2 is middle of cells	 1.1

			 1.2
polycaprolactone + Argon 2	Contaminated therefore discarded		No image
polycaprolactone + Argon 3	Contaminated therefore discarded		No image
polycaprolactone + ammonia 1	30 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	confluent all the way	 1.1  1.2
polycaprolactone + ammonia 2			No image

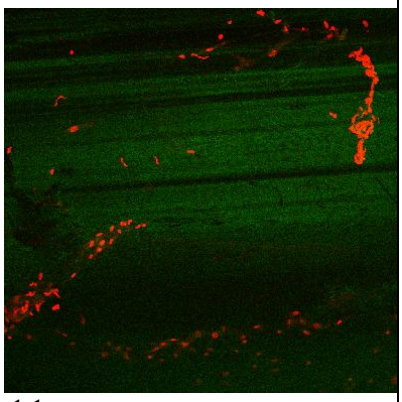
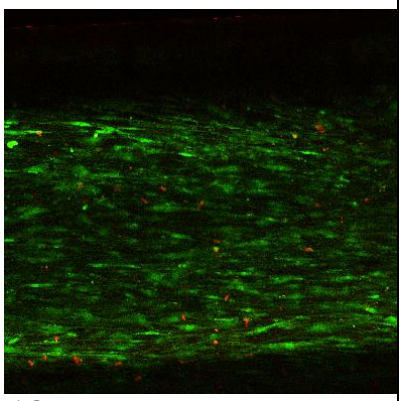
polycaprolactone + ammonia 3	30 mm (lots of attachment both ends, all the way. (Cells growing on capillary tube + cells growing on tissue culture flask). Media turned orange	Cells grew all the way. Mostly confluent. 1.2 is healthy bit.	 3.1  3.2
Solanyl 2% Chitosan 2	30 mm Lots of cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	16 definitely cells, a lot (optical microscope)	No image
Solanyl 2% Chitosan 3	0 mm. Media remained red	no visible cells	No image
Solanyl + Chitosan 1	Few cells growing on capillary tube + cells growing on tissue culture flask, probably 0. Media turned orange	4 or 5 clusters of cells, nothing directly measurable	 1.1

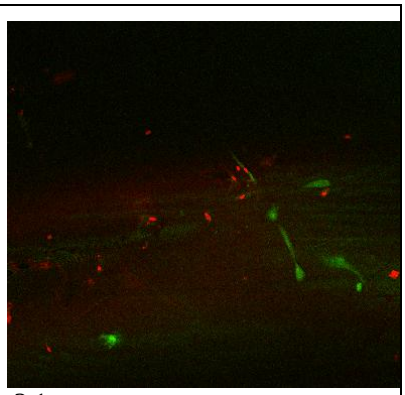
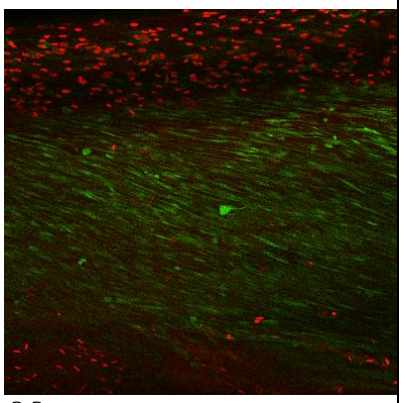
			 1.2
Solanyl + Chitosan 2	Few cells growing on capillary tube, lots of floating cells, probably 0. Media turned orange	no visible cells	No image
Solanyl + Chitosan 3	17.5 mm in clusters (6.3, 4& 4.5 mm) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Clusters of cells	No image
Solanyl + Argon + Chitosan 1	Nothing visible. Media remained red	no visible cells	No image
Solanyl + Argon + Chitosan 2	4 mm in clusters (1.5, 0.5, 2 mm) + cells growing on capillary tube. Media turned orange	Clusters of cells	 2.1

			 2.2
Solanyl + Argon + Chitosan 3	26 mm in two clusters (8, 2mm gap then 18 mm) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Cells visible all the way along sample.	 3.1  3.2
Solanyl + ammonia + Chitosan 1	12-30 mm + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Cells visible all the way along sample with little gaps probably from removal from glass tube)	No image

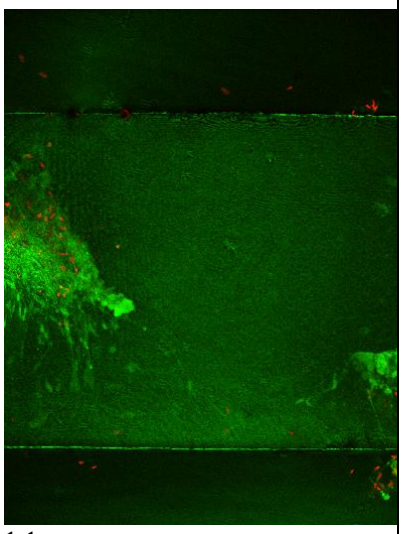
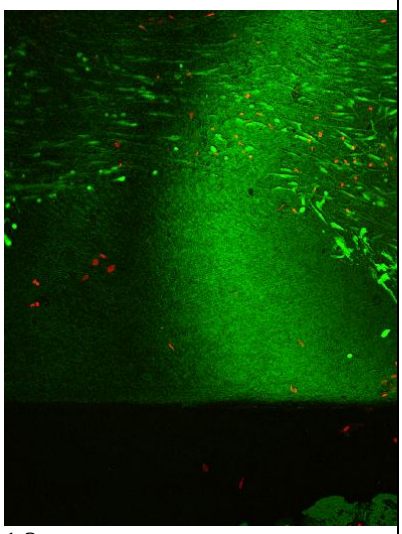
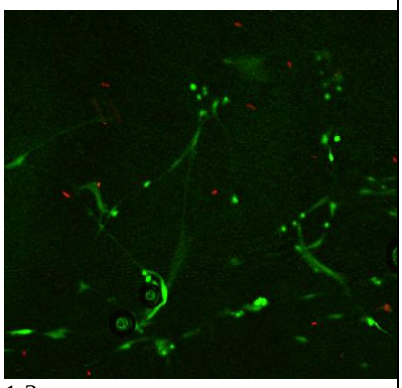
Solanyl + ammonia + Chitosan 2	30 mm, lots of cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Cells visible all the way along sample with little gaps probably from removal from glass tube)	 2.0
Solanyl + ammonia + Chitosan 2	30 mm lots of cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Cells visible all the way along sample with little gaps probably from removal from glass tube)	No image

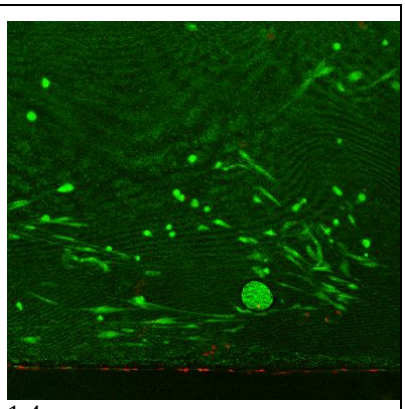
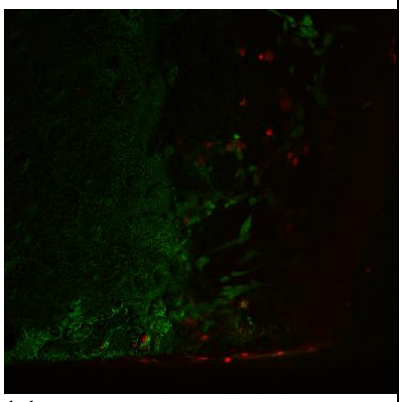
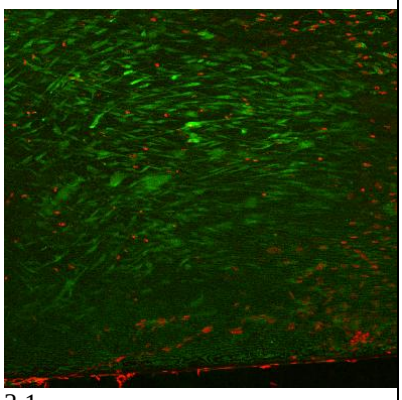
Day	0	29	30	
Date	25/08/2005	23/09/2005	24/09/2005	No image
Solanyl 1		Sample detached at one end. Cells growing all the way along sample. Cells at both ends, + cells growing on capillary tube + cells growing on tissue culture flask Media remained red	Confluent for all but last part of sample. Still plenty of cells, just not all the way. Possibly ripped off.	No image
Solanyl 2		Cells present on detached end, not much else + cells growing on capillary tube + cells growing on tissue culture flask. Media remained red	no visible cells	No image
Solanyl 3		Contaminated therefore discarded		No image
Solanyl + Argon 1		Contaminated therefore discarded		No image
Solanyl + Argon 2		Contaminated therefore discarded		No image
Solanyl + Argon 3		Contaminated therefore discarded		No image
Solanyl + ammonia 1		Contaminated therefore discarded		No image
Solanyl + ammonia 2		Contaminated therefore discarded		No image
Solanyl + ammonia 3		Contaminated therefore discarded		No image
Solanyl 2% Chitosan		Contaminated		No image

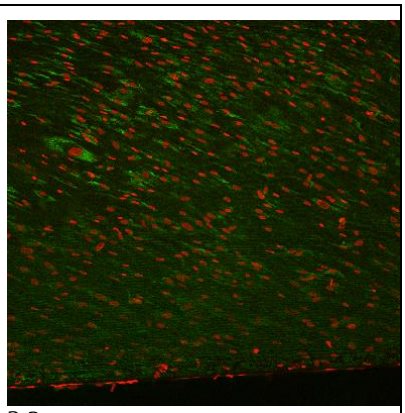
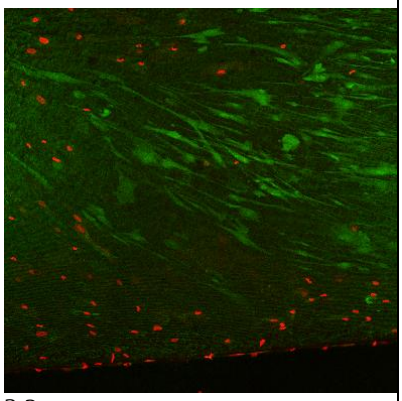
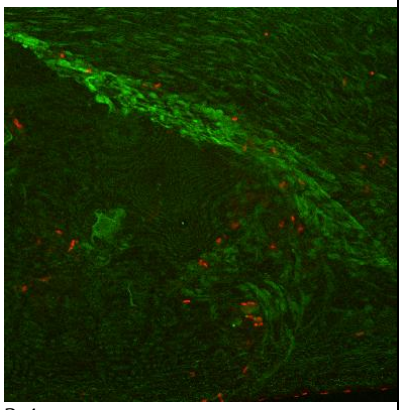
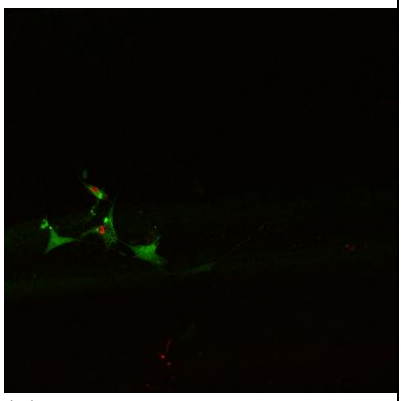
1	therefore discarded		
Solanyl 2% Chitosan 2	Contaminated therefore discarded		No image
Solanyl 2% Chitosan 3	Contaminated therefore discarded		No image
Solanyl + Chitosan 1	4mm gap, 2mm, 2mm gap, 2mm, 2mm gap, 2mm. 4mm other end + cells growing on capillary tube + cells growing on tissue culture flask . Media turned orange	Picture 1.1 is dead bit, 1.2 is part of confluent bit.	 1.1  1.2
Solanyl + Chitosan 2	0 mm. Media remained red	no visible cells	No image
Solanyl + Chitosan 3	0 mm. Media remained red	no visible cells	No image
Solanyl + Argon + Chitosan 1	0 mm. Media has remained red	no visible cells	No image

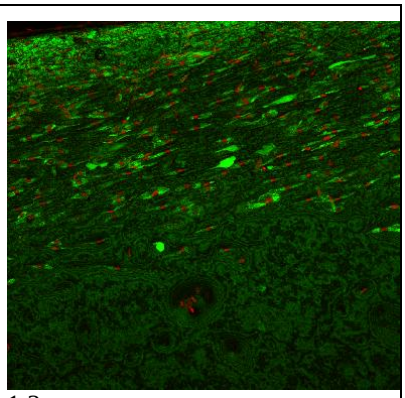
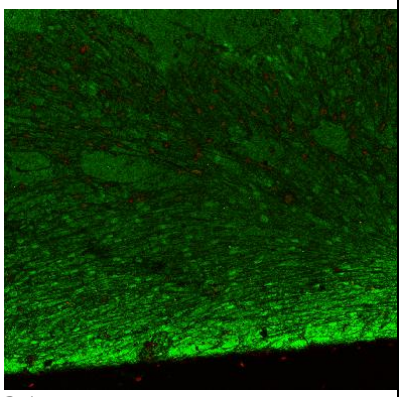
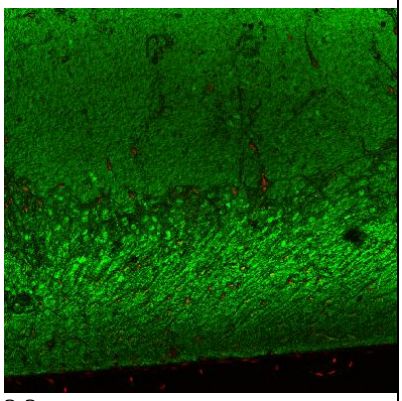
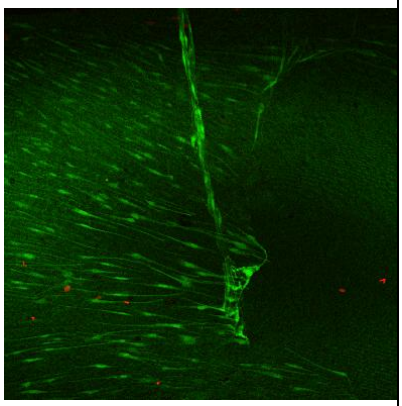
Solanyl + Argon + Chitosan 2	8 mm (8mm dense, then can't see well enough. Probably a lot more) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange /red	Mostly confluent (2.1 is loose bit, 2.2 is confluent bit.	 2.1  2.2
Solanyl + Argon + Chitosan 3	30 mm confluent all the way. Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange /red.	Confluent all the way.	No image
Solanyl + ammonia + Chitosan 1	0 mm. Media remained red	No visible cells.	No image
Solanyl + ammonia + Chitosan 2	30 mm (confluent all the way I think, pretty sure) + cells growing on capillary tube + cells growing on tissue culture flask. Media is orange/ red.	3x2mm gaps in confluence. Still cells. Probably due to removal. (Picture 2.2, cells conf)	No image
sol + ammonia +	0 mm. Media	no visible cells	No image

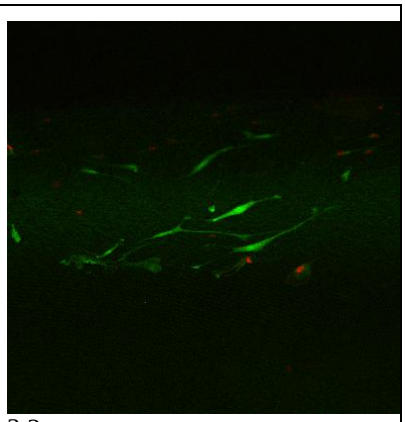
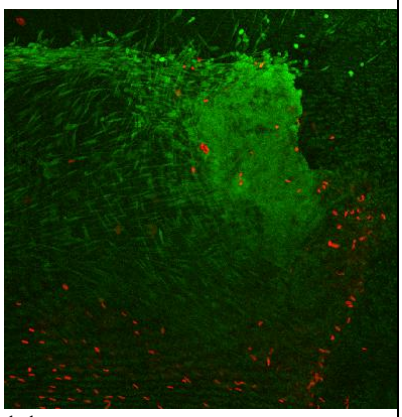
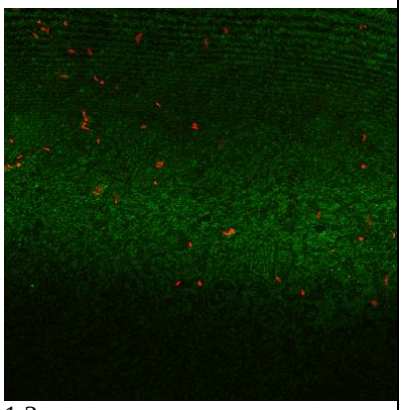
Chitosan 2	remained red		

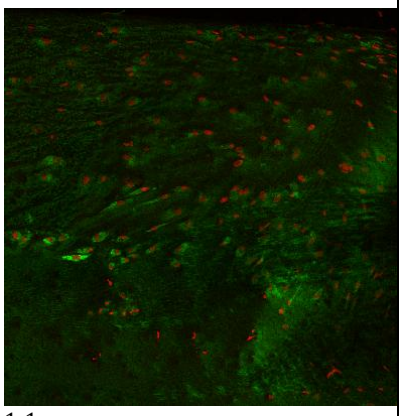
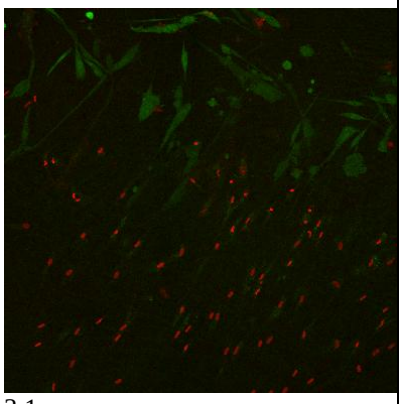
Day	0	29	30	
Date	25/08/2005	23/09/2005	24/09/2005	
Polyurethane 1		25 mm (5 mm gap, 2mm from one end. Rest is confluent. Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	1.1 is one end. Patchy like 1.1 for 4mm, gap for next 10mm, patchy for last 16mm. Confluent and loose bits like 1.2, 1.3 & 1.4	 1.1  1.2  1.3

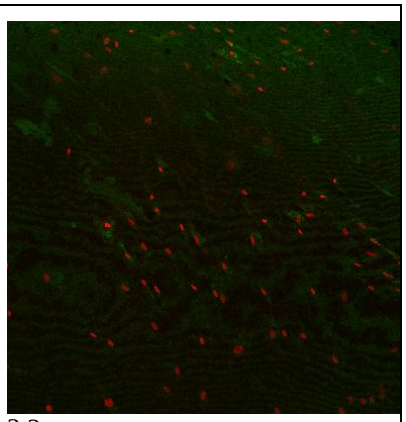
			 1.4
Polyurethane 2	0 mm. Media remained red	no visible cells	No image
Polyurethane 3	0 mm. Media remained red	no visible cells	No image
Polyurethane + Argon 1	2mm + cells growing on capillary tube, no cells growing on tissue culture flask. Media remained red	2mm	 1.1
Polyurethane + Argon 2	0 mm. Media remained red	no visible cells	No image
Polyurethane + Argon 3	30 mm (confluent all the way.) Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Confluent except for rips. Live and dying bits. 3.1 has both, 3.2 is dying bit, 3.3 is clear bit	 3.1

			 3.2  3.2  3.4
Polyurethane + ammonia 1	5.25 mm (4mm confluent. Other end, 2mm gap, 0.75mm, 0.5mm gap, 0.5mm) + cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	1.1 is isolated patch at one end. Like 1.2 for first 6mm other end. Cells only 1/3 way up width. Probably the strip was tight	 1.1

		against the tube prohibiting further spreading.	 1.2
Polyurethane + ammonia 2	30 mm (confluent all the way.) cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	All the way. Mostly confluent. Gaps both sides, but cells on both sides. All the way. 2.2 is gap/ ripped bit	 2.1  2.2
Polyurethane + ammonia 3	30 mm (confluent all the way.) + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	confluent for first 7mm, 4mm ripped but with cells, 2mm confluent, 14mm mostly empty/ few cells	 3.1

			 3.2
Polyurethane + Argon + Chitosan powder 1	Can't measure accurately. Plenty of cells, but some ripped off from moving flask. + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	Mostly empty. Either poor growth or lost due to ripping. 1.1 is best bit; the rest is almost empty/ scattered. 4x2mm one at each end, 1.1 and other dying bit. Mostly empty. Rest like 1.2 at best.	 1.1  1.2
Polyurethane + Argon + Chitosan powder 2	0 mm. Media remained red	no visible cells	No image
Polyurethane + Argon + Chitosan powder 3	0 mm. Media remained red	no visible cells	No image
Polyurethane + Argon @ Galashiels	0 mm. Media remained red	no visible cells	No image

1			
Polyurethane + Argon @ Galashiels 2	0 mm. Media remained red	no visible cells	No image
Polyurethane + Argon @ Galashiels 3	0 mm (little cells growing on capillary tube & cells growing on tissue culture flask). Media remained red	no visible cells	No image
Polyurethane + Oxygen @ Galashiels 1	12 mm + (12mm confluent/ patchy. 6mm gap, then can't tell. Plenty on other end.) + Cells growing on capillary tube + cells growing on tissue culture flask. Media turned orange	4.5mm confluent to not (1.1 is bloody unclear.), 6mm form other end is 3mm cluster. Poorly stained.	 1.1
Polyurethane + Oxygen @ Galashiels 2	0 mm (2-3cells on flask, little + cells growing on capillary tube. Media turned orange	no visible cells	No image
Polyurethane + Oxygen @ Galashiels 3	30 mm (all the way confluent except 2mm patchy 2mm from one end). Media turned orange	28 (2mm gap 2mm from one end. Some cells still alive 8hrs after staining)	 3.1

			 3.2

Day	0	29	30	
Date	25/08/2005	23/09/2005	24/09/2005	
Polylactic acid 1		Can't measure. (Free floating. Both ends have cells. Few cells growing on tissue culture flask, + cells growing on capillary tube. Media turned orange	confluent all the way	No image
Polylactic acid 2		Cells on one end contracting sample. Can't measure. Probably not on other end. Cells growing on tissue culture flask. Media turned orange	11mm. Confluent to not dense. 1.1 is healthy bit, 1.2 where cells have started dying	No image
Polylactic acid 3		0 mm. Media remained red	no visible cells	No image
Polylactic acid + Argon 1		0 mm. Media remained red	no visible cells	No image
Polylactic acid + Argon 2		0 mm. Media remained red	no visible cells	No image
Polylactic acid + Argon 3		0 mm. Media remained red	no visible cells	No image
Polylactic acid + ammonia 1		0 mm. Media remained red	no visible cells	No image
Polylactic acid + ammonia 2		0 mm. Media remained red	no visible cells	No image
Polylactic acid + ammonia 3		0 mm. Media remained red	no visible cells	No image

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