The Sensitivities-Enhanced Kriging method

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IMBIOTOR
Control oriented investigation of tissue engineering of cartilage

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Contents

Summary

This report is about the European IMBIOTOR-project. The aim of the project is to develop an intelligent bioreactor to control the growth of tissue-engineered cartilage. The project will run for three years, leading to a prototype bioreactor. This report describes an control oriented investigation of tissue engineering of cartilage in a bioreactor.
The first phase in the controller design process is product and process investigation.The product to be produced in the bioreactor is tissue-engineered cartilage. The main constituents of cartilage are cells (chondrocytes), extracellular matrix (collagen, proteoglycans) and water. The chondrocytes produce extracellular matrix. This extracellular matrix with bounded water is responsible for the tissue properties.The process to be controlled is the production of tissue-engineered cartilage out of chondrocytes seeded on a biodegradable scaffold material. This scaffold with seeded chondrocytes is placed in a fluid in the bioreactor. The chondrocytes are supplied with nutrients and influenced by chemical and mechanical factors. The chondrocytes produce neo-tissue which in combination with the scaffold material needs to have sufficient mechanical integrity to function in vivo.Because tissue engineering is a new research area, not much is known. The main literature available on this subject belongs to G.Vunjak-Novakovic et al. They describe the influence of variables, like cell seeding, mixing intensity, microgravity and gaseous exchange. Their information is based on experiments and everything is interpreted physically. The aim of their research is to improve their understanding of tissue development, not to develop a control system. The specialists are occupied in their specialistic area and there is no overall view of the process, which is necessary for modelling and controlling it. Producing engineered tissues is based on knowledge obtained from previous experiments. Nowadays the implants are produced by recipes and are based on experiences of the past, which comes down to feed-forward control combined with simple feedback controllers to keep several process parameters constant.

This report contains lists of inputs and outputs, which are a first step in the design of a control system and they offer a overall view of the process. Also, optimization criteria are defined to optimize the process: *Designing an intensive bioreactor control system with operation strategies which minimize production time and maximize the tissue function and the cell viability. The created implant needs to have sufficient mechanical integrity to function in the human body.*
The controlled outputs, described in section 4.4.1, are derived from these optimization criteria. These outputs are to be controlled to obtain optimal production of tissue-engineered cartilage. Not all controlled outputs can be measured, therefore information is obtained by measuring several other outputs. All outputs that are measured are called measured outputs and are described in section 4.4.2. With estimators and these measured outputs internal variables can be computed to give a better view of the current state of the process. These internal variables are described in section4.4.3. With the information of measured outputs and internal variables the control inputs, section4.4.6, can be determined. These are inputs that can be manipulated during control to effect the outputs. The aim of controlling is that the controlled outputs gradually reach the desired value. Not all inputs can be controlled. The inputs that can not be controlled are called disturbances. They contribute together with the prescribed trajectory to the exogenous inputs, describe in section 4.4.5. Before starting the production of tissue-engineered cartilage the initial conditions has to be determined. These

conditions partly determine the system behaviour. Some conditions are prescribed, while others are given. In controlling the most difficult problem is to determine the current state of the process. This problem is overcome once a suitable process model is found as well as an adequate estimator for determining non-measurable variables.

The main advantage of control theory is its applicability for many types of systems. Investigation in the comparable processes, like culture of animal cell, bioremediation, fermentation, waste water purification, plant cell growth, culture skin grafts, vaccine production, animal cell technology and solid-state fermentation, offered different process models, estimators and controllers, as mentioned below.

- Process descriptions: PDE in combination with ODE, Neural networks, AR modelling, sequential modelling, time-varying modelling, difference equation model, first order dead time transfer function, optimal system (state system + adjoint system), linear dynamic model, (non) segregated models and approximate nonlinear model
- Estimators: Kalman filters, genetic algorithm, Luenberg/Kalman observers, asymptotic observers(state estimation), observer based estimators (parameter estimators), multi rate adaptive estimator, network based estimator, H_∞ based estimator.
- Controllers: feedforward controllers, PID controllers, self organizing fuzzy logic controller (SOFLC), variable structure control (VSC), state feedback controllers

This investigation showed that bioprocesses are complex processes to model and to control, due to their nonlinear behaviour, multiple in- and outputs, and non-measurable outputs. In literature several methods are described to model and control comparable processes. It has to be stated that in these articles the application of the methods are well described, but these articles do not offer a comparison with other methods. Concluding the results found in literature there is no universal best method for modelling and controlling bioprocesses. The investigated literature emphasizes process modelling, and process controlling is only slightly described. The applied controllers are simple controllers.
The models and controllers in literature are based on measurement data. So it will be necessary to obtain data, to create a model and control system.

The process in a bioreactor can also be compared to chemical processes. Control design is an already much investigated topic in the chemical industry. For slow chemical processes model based control is often used. By trying to develop a model based control system interaction between the specialists is stimulated. This interaction may result in a better understanding of the process. By designing a model based controller the designed model is verified with reality.
The European project is in its first phase and the workpackages in the project are executed parallel instead of sequential. As of yet no model is available, which makes it difficult to design a model based controller.

Chapter 1

Introduction

Engineering of tissue(s) (e.g. cartilage, bone, blood vessels) could form the basis for on the one hand new therapies for patients suffering from the loss of tissue or its function and on the other hand in vitro studies of normal tissue. One approach towards the development of functional tissue equivalents is the bioreactor cultivation of isolated cells on biodegradable polymer scaffolds. The scaffold provides a defined, three dimensional structure of cell attachment and tissue organization. The bioreactor provides control over the hydrodynamic and biochemical factors in this cell environment. The tissue-engineered cartilage construct resembles natural cartilage and continues to remodel as a result of in vivo implantation.
This report is about the European IMBIOTOR-project. The aim of the project is to develop an intelligent bioreactor to control the growth of tissue-engineered cartilage. The project will run for three years, leading to a prototype bioreactor, the IMBIOTOR. The project is well grounded in both the scientific and industrial communities with six participants with complementary multidisciplinary skills.
The project is divided into several workpackages. Each workpackage has its own tasks, deliverables and participants. The IMBIOTOR project will be described in more detail in Chapter 2. This report is about the development of the control part of the IMBIOTOR, which is part of workpackage 6. The central topic of this report is a control oriented investigation of the production of tissue-engineered cartilage in a bioreactor. It has to be stated that this report does not contain a controller design for the optimal production of tissue-engineered cartilage.
Before being able to design a controller for the production of tissue-engineered cartilage, further knowledge of cartilage and tissue-engineered cartilage is needed (Chapter 3).
Secondly, the production process has to be investigated. With this information an optimization criteria is defined and lists of inputs and outputs are set up (Chapter 4).
The main advantage of control theory is its applicability for many types of systems. Thus, it is useful to look for comparable process control systems before designing the control system for the production of tissue engineered cartilage. Comparable processes are animal cell culture, bioremediation, fermentation, waste water purification, plant cell growth, culture of skin grafts, vaccine production, animal cell technology and solid-state fermentation. (Chapter 4)
Finally, the main results will be summarized in the form of conclusions in Chapter 5. In Chapter 6 a summary of the report is given.

Chapter 2

The European project

2.1 Problem description

Injuries and degenerative diseases to load-bearing soft tissues are extremely common in hospital clinics and involve all ages of population. As an example, rupture of the anterior cruciate ligaments (ACL) accounts for 50% of all knee injuries in young and active subjects. Once a knee joint is ACL deficient, its instability usually leads to meniscal tearing and damage to the articular surfaces which may develop into early osteoarthritis. The vascularity of many soft tissues is poor, as is the case for many ligaments and tendons, or non-existent as with articular cartilage. Therefore the natural healing response is limited. Thus there is a major requirement for repair strategies, which will restore functionality to the load bearing tissues. Soft tissue replacements amount to an estimated 35% of the world market for all medial devices. Tissue engineering provides a major focus for developments within this area due to the relative failure of graft and prosthetic-based strategies.
Tissue engineering is a major research area of biotechnological research today and it is expected that this type of technique will ultimately transform medical practice. The tissue engineering approach used for this European project is deduced from the approach, adopted by ATS Inc. and other tissue engineering companies, involving autologous or allogenic cells which are seeded onto a biomaterial scaffold, then processed in vitro to induce the fabrication of neo-tissue. Ideally the scaffold material should possess mechanical integrity and be resorbable ensuring transfer of functionality post-implantation from the scaffold to the cell-derived neo-tissue.

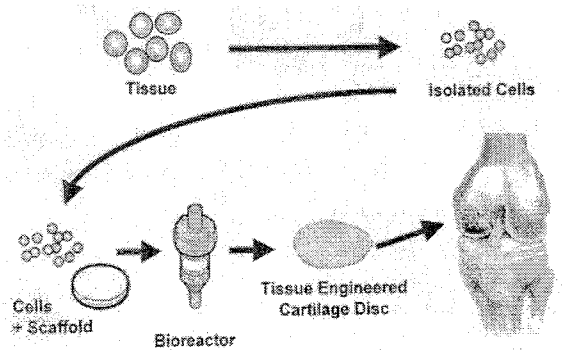

Figure 2.1: *Cartilage tissue engineering approach used for the european project*

The success of such implants is dependent on the induction of cells to proliferate and synthesize extracellular matrix at an appropriate rate and with sufficient organization to ensure functionality. Futhermore, tissue engineered implants must be produced in a highly controlled and aseptic manner.

Tissue processing requires the application of both biochemical and biophysical stimuli for induction of appropriate and enhanced cellular growth, biosynthesis and tissue remodelling. Moreover, nutrients and waste products need to move freely through the cellular constructs to minimize the presence of regions with necrotic cells. Inadequate tissue culture conditions are currently a major problem, with constructs of greater than 2 mm in thickness containing large necrotic regions. Futhermore, differences in the inherent metabolic characteristics of cells isolated from different patients will lead to variability between end-point implants. In addition storage, handling and applications in a clinical environment poses significant hurdles, as live tissues can not easily be moved.

Bioreactor are machines to provide the transport system for nutrients to cells and allow the efficient withdrawal of toxic or inhibitory metabolic by-products. They have been developed for a range of biotechnological applications. An appropriate bioreactor is required to enhance both the

- efficacy: the development of appropriately functional neo-tissue for a specific application

- efficiency of manufacture: associated with the overall cost of production and therefore related to rate of neo-tissue formation, degree of automation and quality assurance.

2.2 Project description

The aim of the European project is:

to develop a prototype intelligent bioreactor (IMBIOTOR) to improve the efficacy and efficiency of manufacturing tissue engineered implants for the repair of load-bearing musculoskeletal tissues.

IMBIOTOR will be developed to apply both biochemical and biophysical stimuli required for optimized tissue growth and remodelling in a highly controlled manner. Potential benefits are that the bioreactor will be able to provide an enhanced environment for neo-tissue formation compared to existing systems and therefor improve both the efficacy and efficiency of production of tissue engineered implants. The novelty will be in the use of local and bulk changes in the construct, to change the input parameters to influence the overall performance. The project will run for three years, leading to a prototype IMBIOTOR. The project is well grounded in both the scientific and industrial communities with highly competent participants with complementary multidisciplinary skills.

2.3 The partners

The participants of the European project are listed below.

Nr.	Org.	Co.	Business Activity/ Main Mission/ Area of Activity	RTD Role in Project
1	QMW	UK	Specialist tissue engineering and medical engineering research.	Mechanotransduction, influence of mechanical conditioning. Development of on-line biosensors. Management of project
2	DIMP	I	Specialist in biomaterials and methods to quantify and improve delivery of solutes to biological tissues.	Polymer testing techniques. Quantification and description of fluids, macromolecules transport within cellular constructs
3	TU/e	NL	Specialist biomedical engineering technology centre associated with mechanical engineering faculty. Includes both materials technology group and control and process engineering group.	Development and validation of theoretical model. Development of control system for bioreactor.
4	VTSI	G	Major European tissue engineering company.	Design and Assessment of bioreactor development and commercial input.
5	Imedex	F	Collagen manufacturer.	Exploitation and dissemination of the acquired knowledge.
6	LEBAO	G	Laboratory involved in biotechnology and the generation of artificial organs, generating innovations in bioreactors.	Design, production and evaluation of intelligent bioreactor.
Sc 1	Intospace	G	Exploitation manager.	Exploitation and dissemination of the acquired knowledge.

(*Partners involved in the IMBIOTOR project*)

- QMW: Interdisciplinary Research Centre in Biomedical Materials, Queen Mary & Westfield College, University of London, UK (London)
- DIMP: Department of Materials and Production Engineering, University of Naples, ITALY (Naples)
- TU/e: Department of Mechanical Engineering, University of Technology Eindhoven, THE NETHERLANDS (Eindhoven)
- VTSI: Verigen Transplantation Service International, GERMANY (Leverkusen)
- IMEDEX: Imedex Biomateriaux, FRANCE (Lyon)
- LEBAO: Leibniz Laboratories for Biotechnology and Artificial Organs, Medical School, Hannover, GERMANY (Hannover)

2.4 Distribution of tasks

The project is separated in several workpackages, shown in figure 2.2. Each workpackage (WP) has its own tasks, deliverables, participants and time span.

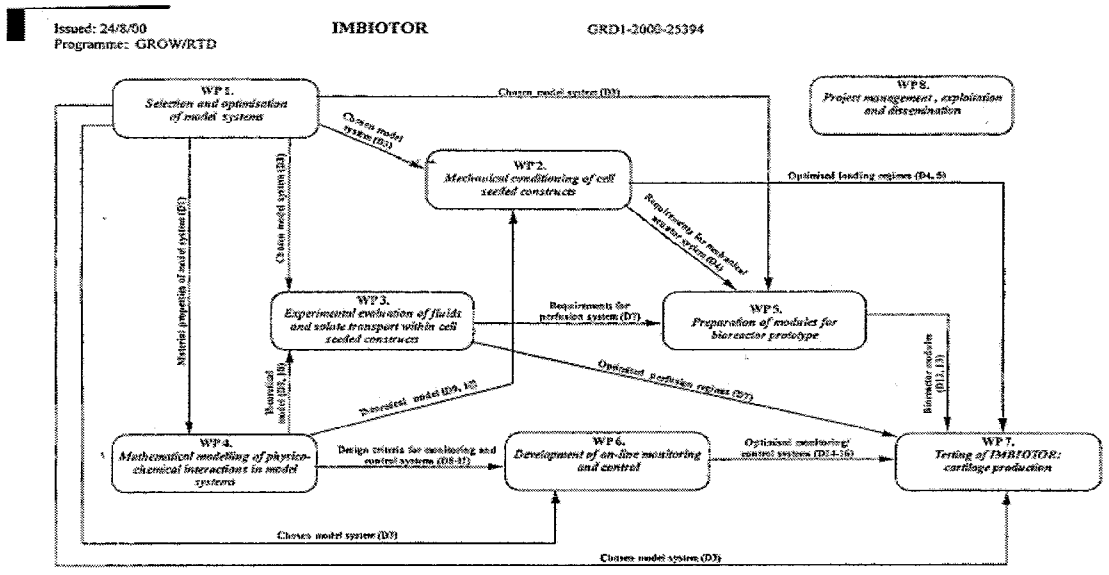

Figure 2.2: *Distribution of tasks over the workpackages*

2.4.1 The work plan for the project

The work plan for the project is divided into two phases, which will run in parallel for a large proportion of the project. Phase 1 will develop and characterize representative model systems and identify environmental input parameters which influence neo-tissue formation in a beneficial manner. Selected model systems, manufactured and supplied by Imedex Biomateriaux, will be characterized using established techniques of QMW and DIMP. The model systems will involve combinations of cell and material components which represent state-of-the-art tissue engineered implants (WP1). These model systems will be utilized, in experimental tests, to identify dynamic mechanical loading regimes which are beneficial to the development of neo-tissue through mechanotransduction pathways (WP2). The stimulus response (bulk biochemical response to mechanical stimulations) from constructs will be evaluated, the results providing input into WP4. Futhermore the transport of fluids, nutrients and active biomacromolecules will be investigated both experimentally and theoretically (WP3). The final aim is to define a mathematical model able to describe fluid-dynamic performance of the bioreactor and therefore the profiles of nutrients and biological active macromolecules within the cellular construct (WP4). This will bring together the complementary skills from QMW, DIMP and TU/e. This mathematical model will represent the "intelligence" of the bioreactor and will be used for the operational control of the bioreactor and determine homeostatic feedback strategies. Key deliverables from phase 1 will be the identification of optimized input parameters which will enhance the quality and rate of development of neo-tissue and thereby contribute to the efficiency of production of tissue engineered implants.
Phase 2 (WPs 5-7) will utilize the deliverables from phase 1 in the design and manufacture of a prototype production equipment for fabrication of tissue engineered implants against specified design criteria. These criteria are set as follows:

- Fully or semi-automated control
- Maintenance of a closed aseptic environment during manufacture

- Intelligent control incorporating novel monitoring systems for biological output and the relevant feedback mechanisms necessary to provide optimum stimulation and ensure uniformity of the end-product.

WP5 will initiate and test mechanical design concepts which will be incorporated into the prototype device. In general, bioreactor technologies have been employed previously, to facilitate scale-up of cell culture for recombinant protein manufacture and waste/pollution control while maintaining control and sterility. The challenges of the current project are somewhat different, in that scale up is not a factor. Indeed for autologous cell-seeded devices, cell numbers may be as low as 1 x 10^6. Maintenance of sterility and automated control on a micro scale are therefore key. Controlled systems for medium delivery/extraction, to maintain and control perfusion of medium through the devices, and a mini-actuator system for mechanical stimulation will be developed. During WP6 appropriate sensor systems for on-line monitoring of input-parameters, known to influence cellular activity, and output parameters, indicative of the construct performance, will be sourced or developed. The efficacy of the sensors will be experimentally tested using the model systems characterized in WP1. Sensor systems should have sufficient resolution to detect subtle changes in input or output parameters associated with altered cellular activity. Futhermore the sensors must be sufficiently practical for use in a commercial bioreactor system. Examples of sensor systems which may meet the defined criteria include the Ultra-P micro flow-through electrodes for pH, pO_2, pCO_2 and temperature. Novel non-invasive methodologies for direct or indirect assessment of cellular activity will be developed. Futhermore, feedback control mechanisms will be developed to allow alteration of input parameters in an appropriate manner in response to the output measures (WP6).

The deliverables from WPs 2-6 will be applied to the manufacture and testing of the prototype IMBIOTOR (WP7). The prototype will be tested using a model system characterized in WP1 and incorporating beneficial input parameters identified in WP2 and 3. The efficacy and efficiency of production of tissue engineered devices using the bioreactor will be compared with conventional static culture systems and with currently available commercial systems, such as the rotating wall bioreactor, which does not incorporate feedback control. Cartilage is selected as the tissue for testing the IMBIOTOR prototype, based on the existing expertise of all of the academic partners and the product base of the industries. It represents a specific engineering plant, whose production can be completed within the time frame of the project.

2.4.2 Workpackage 6

Development of on-line and/or integrated monitoring and control mechanisms. There are three possible methods for the determination of cell activity and construct performance, namely:

- Whole or partial sampling of construct. This involves the removal of all or part of a construct, for subsequent analysis and can not be performed real-time. This represents the current state of technology for many tissue engineered implants.

- Sampling of medium allows for the constructs to be left in place and intact, although there are problems associated with the relationship between elements present in the medium compared with that in the construct itself.

- In situ construct testing involving a real-time on-line monitoring system

A number of parameters will be considered for the successful implementation of the intelligent bioreactor, these include:

pH	pO_2, pCO_2
Proteoglycan synthesis	Collagen synthesis
Interleukins	Enzymes such as matrix metalloproteinases (MMPs)
Structural stiffness	Permeability
Cell viability	Cell number

(*Parameters for controlling a bioreactor for production of articular cartilage*)

O_2 and nutrient pulsing will be applied, and the response will be monitored to establish cell growth and viability within the bulk constructs. Many of these parameters are inter-related. For example, the structural stiffness of the developed construct will be a function of both the synthesis and breakdown of extracellular matrix, the latter determined by the regulation of proteolytic enzymes. DIMP has experience with fluorescently based methods to measure non-invasively pO_2 and pH. Disposable, low cost needle-type sterilized enzyme electrodes for glucose, lactate and pyruvate will be used. All have been developed using oxidase enzymes and classical reaction chemistries of peroxidase detection. This is an established monitoring route adapted for use in undiluted samples exploiting biocompatible, polymeric protective membrane barriers. Similar commercial and in-house electrodes for O_2, CO_2 and NO will be used. The unique feature of this approach will be an intermittent calibrant flow over the sensors in situ to ensure reliable operation. The calibration will first be established manually, and then be integrated with the automated actuator arm of IMBIOTOR.
Bioreactor intelligent control systems include both open-loop (feed-forward) and closed-loop (feedback) components. With feed-forward (i.e. steering) all relevant a priori known information can be used to determine appropriate levels of manipulated input signals (environmental and mechanical). Additional intelligence, such as determined by specific tissue characteristics or learned behaviour from previous growth experiments, can also be used in the open-loop situation. Reasons to use feedback are robustness for changes and uncertainties in tissue parameters and models, and rejection of environmental disturbances affecting cell growth.
Before designing the actual control parameters, the control structure should be investigated. This involves establishing which inputs are most effective, and which outputs can be measured, and are most suited to be used for control. From the relevant environmental parameters (nutrients, oxygen, CO_2, pH, temperature) and mechanical parameters (applied strain, frequency), an input selection procedure should reveal the most effective control inputs. The output selection comprises also the determination of relevance of distributed measurement systems versus accumulated sensor information. The input/output selection should be performed using extensive model knowledge, gathered in previous workpackages, in particular WP4. The control design (feed-forward and feedback) consists of model-based optimization of controller parameters, including modelling of specifications and disturbances. The growth rate of the tissue and possible dynamics of the interactions between the control inputs and the measured outputs will also be assessed. It should be noted that the design of the control system starts in the modelling phase, and close interaction with relevant other workpackages is essential. Finally, the control design will be implemented, and fine tuned using extensive experiments and identification of crucial model parameters.

2.4.3 Problem formulation for this report

Tissue engineering is a new, young research area. The development of cartilage tissue, out of autologous or allogenic cells which are seeded on a biomaterial scaffold and then placed in a bioreactor, is very premature. The information and knowledge available of this process is limited, and the numeric model is under construction. Most of the information is obtained from experiments. The process of cartilage production is slow and complex.
The aim of this report is
to investigate the possibilities to control the process of cartilage neo-tissue formation in a bioreactor, using a literature survey in comparable processes.

Before the process can be controlled, the process and the product cartilage need to be understood. This requires literature investigation. The aim of building a controller is to control and optimize the process. Therefore it is necessary to define an optimization criterium. Subsequently the inputs and outputs of the process have to be defined. Actually, not all outputs can be measured. The non-measurable outputs can be computed by a control algorithm and are called internal variables.
Beside the actual primary control system design, literature investigation is done to find control schemes for comparable processes, like culture of animal cell, bioremediation, fermentation, waste water purification, plant cell growth, culture of skin grafts, vaccine production, animal cell technology and solid-state fermentation. These control schemes might be useful for the actual controller design.
It has to be stated that this report does not contain a controller design for the optimal production of tissue-engineered cartilage.

Chapter 3

Cartilage

Cartilage is a miraculous type of connective tissue. It consists for a large amount of intercellular water and still it is capable to resist large forces. The patella consists of 75-78% of water. Strength and elasticity are the main properties of cartilage. These properties are owed by the special manner in which the water is bounded. The information on cartilage is mainly obtained from [Morree, 1996] and [Bader and Schechtman, 19....] .

3.1 General properties

There are three types of cartilaginous soft tissues found in the body, namely elastic cartilage, hyaline cartilage and fibrocartilage. The articular cartilage, which is located in synovial joints, consists of hyaline cartilage and is the focus of the European project. It is the soft tissue, which covers the articulating ends of the bones, which terminate at a synovial joint. It can also be found as protective cartilaginous rings in the trachea and in the larynx and as connective tissue between ribs and sternum. The tissue has a transparent white color with a blue glow. Under the light microscope the tissue has a homogenous structure, but using electron-microscope the collagen fibres are clearly visible.
Articular cartilage in the synovial joints is subjected to forces which pass through the joint. Cartilage has to reduce the contact stresses to safe values. Thus protect the chondral bone from damage and it has enable movement. So cartilage has to be elastic, shock-absorbing and needs to have a smooth sliding surface, with low-wear and low-friction bearing surfaces. Articular cartilage in conjunction with its lubricant, synovial fluid, produce a coefficient of friction in normal healthy joints which is lower than can be achieved with a man-made engineering system. Some interesting facts about cartilage are shown in the table below.

Magnitude of resultant forces, during the period just after heal strike	
walking	3.500 N
vigorous sporting	10.000 N
Contact stresses	
compressive strength of cartilage	1 - 5 MPa
modulus of chondrocytes	2 kPa
Thickness of cartilage	
load bearings	1.5-3.5 mm
patella	5 mm

(*Interesting facts about articular cartilage*)

Articular cartilage does not contain any blood vessels, nerve endings and lymphatic vessels. Nutrients, supplied

by the surrounding tissues, reach the chondrocytes by diffusion, osmosis and perfusion. The nutrient supply is a very slow process and hence a restriction for the thickness of the cartilage tissue.

3.2 Components

Articular cartilage can be considered as a fibre reinforced polymer gel containing cells, known as chondrocytes. These cells are responsible for the synthesis and maintenance of extracellular components. The proportion of the major extracellular elements are given in table *Relative proportion of non-cellular components in adult human articular cartilage* .

component	wet weight
collagen	15-20 %
proteoglycan	3-15 %
water	65-80 %
non-collagenous proteins and glycoproteins	1 %

(*Relative proportion of non-cellular components in adult human articular cartilage*)

The components' interactions determine the physical and mechanical properties of the tissue.
Under the light microscope, four zones can histologically be distinguished in cartilage, namely the superficial zone, the transition zone, the deep zone and the calcified zone. Each zone has a different composition of chondrocytes, collagen fibres, proteoglycans and water. Considered functionally, cartilage is a unity with on each level different demands.

3.2.1 Chondrocytes

The chondrocytes are responsible for synthesis and maintenance of extracellular constituents of cartilage. Chondrocytes are reported to vary considerably in size with values between 7 to 30 μm in diameter and are contained with spaces called lacunae. The ratio of cell volume to tissue volume is lower than in most tissues, accounting for 1% - 10% of the tissue volume, with a reported mean chondrocyte density of 14.000 cells/mm^3.
The characteristics of chondrocytes change with depth from the articular surface. As mentioned before, four zones can be distinguished. These zones are described below:

Zone	Shape of cells	Orientation
Superficial zone	Discoid	Parallel to the articular surface
Transition zone	Ellipsoidal	With long axes to a range of orientations to the articular surface Large cytoplasmic volumes
Deep zone	Ellipsoidal or Spherical	Cells in groups of four to eight arranged in columns perpendicular to the articular surface Large cytoplasmic volumes
Calcified zone	Hypertrophic	Marking the transition to calcified cartilage

(*Characteristics of chondrocytes classified in four zones*)

The cells in the transition zone and the deep zone have large cytoplasmic volumes. These cytoplasmic volumes contain well-developed endoplasmatic retilica and golgi complexes, which produce the main synthetic activity in cartilage.
The chondrocytes in articular cartilage are supplied by nutrients in the synovial fluid, which are transported across the cartilage surfaces.

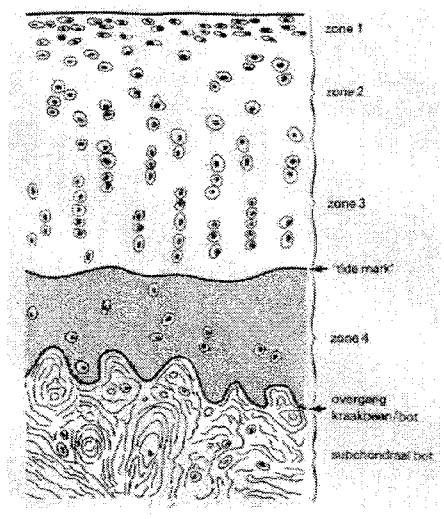

Figure 3.1: *Distribution of chondrocytes in articular cartilage. The boarder between non-mineralized and mineralized is called tide-mark*

3.2.2 Collagen

The building blocks of collagen, the tropocollagen molecule, consists of three polypeptide chains, designated α chains, coiled together in a right-handed helical structure. Each α chain contains approximately 1000 amino acid residues. With the exception of the short non-helical sequences at the end of each chain, one third of the amino acid residues are composed of the small molecule glycine. Of the remainder, approximately 22% are either proline or hydroxyproline, which confer stability to the triple helix due to the inherent rigidity of their ring structures. The precise sequence of amino acids determines the type of collagen present. Nineteen types of collagen have been identified and they are encoded by at least thirty genes. Articular cartilage contains predominantly type II collagen, in which each of three identical polypeptide chains contain a specific content of hydroxylysine residues and linked prosthetic groups.
Like the chondrocytes the collagen fibres vary in shape and orientation with depth below articular surface.

Zone	Shape	Orientation
Superficial zone	Fibrils of 30 nm diameter which are arranged in sheets	The sheets lie parallel to the articular surface in each sheet there is a range of fibril orientations the fibre and fibril bundles are closely packed
Transition zone	Fibrils of 30-80 nm diameter	Fibrils are arranged in a 3D network with larger meshes than in the superficial zone
Deep zone	Fibrils of 30-80 nm diameter	Collagen fibrils surround the columns of cells and thus also tend to be orientated
Calcified zone	Fibrils of 30-80 nm diameter	Collagen fibrils are radially aligned

(*Characteristics of collagen in cartilage classified in four zones*)

The movements in joints take place along load directions. This results in certain preferential load-directions, which are visualized by the distribution of the collagen fibres. The surface of articular cartilage demonstrates directional properties, when examined by a pinpricking technique to induce directional splits. The resulting

split direction is pronounced and generally site specific. The collagen is positioned in the direction of the shear-stresses. When the articular cartilage is loaded, the cartilage in de superficial zone deforms. The collagen-fibrils in this zone have to absorb the shear-stresses caused by the deformation. That's why the collagen fibrils in the superficial zone are positioned parallel to the articular surface.

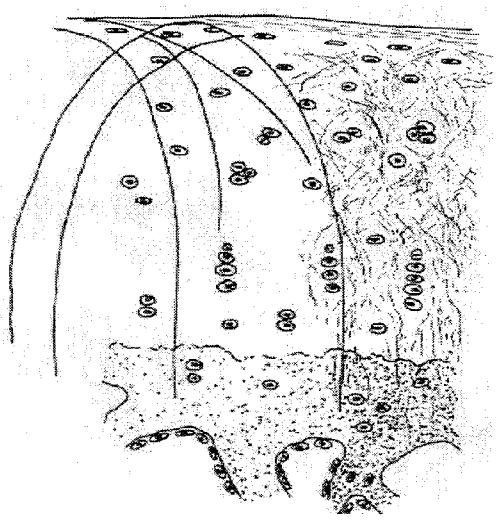

Figure 3.2: *Distribution of collagen in cartilage. On the left side: the main fibril directions, or load directions. On the right side: the position of the collagen fibrils.*

3.2.3 Proteoglycans

The term proteoglycan describes molecules composed of a protein core to which at least one glycosaminoglycan is covalently attached. Glycosaminoglycans (GAG) consist of repeating disaccharide units comprising n-acetyl hexosamine linked to a hexuronic acid or hexose. Several GAGs exist in cartilage, namely chondroitin sulphate (CS), keratan sulphate (KS), heparan sulphate (HS), dermatan sulphate (DS) and hyaluronan. Their relative proportions vary with the type of cartilage and with age.
Proteoglycans are polyanions due to negatively charged sulphate and carboxyl groups associated with KS and CS. These properties control the ability of proteoglycans to bind water osmotically and the polyelectrolyte gels may be considered as osmotic systems. The gel behaves like a solution in tending to dilute itself with solvent. In cartilage this process is limited by the extensibility of the collagen fibres with which the proteoglycans are enmeshed. In this way cartilage can be compared with a sponge.
The proteoglycan content is inhomogeneously distributed throughout the depth of cartilage.

3.2.4 Interstitial water

Cartilage imbibes water due to the hydrophilic nature of the proteoglycans. The highest water content is found near the articular surface and it decreases towards the bone. In the deep zone approximately 65% is water. Most of the water in cartilage is extracellular. About 30% of the interstitial water is associated with the collagenous network. The remaining water is associated with the proteoglycans. This water is freely exchangeable during loading and unloading. It is responsible for the transport of nutrients to the chondrocytes

and the joint lubrication. The tissue fluid contains mobile charged ions, which are responsible for streaming potentials which can alter cell metabolism.

3.2.5 Extracellular matrix

The matrix of proteoglycans, collagen and water outside the cells is called the extracellular matrix (ECM). This matrix differs in composition with the distance from the chondrocytes.

In the immediate neighborhood of the chondrocytes a matrix exists with a large amount of proteoglycans and a low amount of collagen. This matrix is called, the pericellular court. Adjacent to this matrix lies a capsule of thick collagen. This capsule, which is called the pericellular capsule protects the chodrocytes. The largest concentration of collagen fibrils is located on the side of the articular surface.

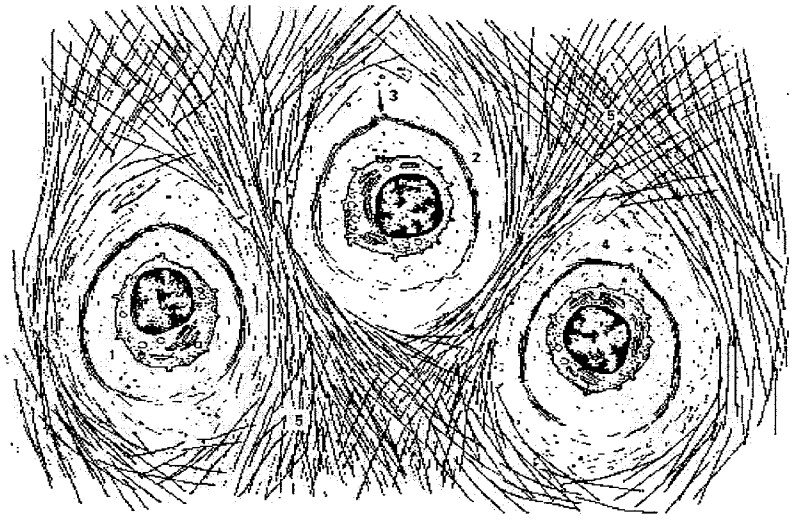

Figure 3.3: *Schematic drawing of articular cartilage in which the special order of chondrocytes is represented. The distincted areas are: 1) pericellular court 2) pericellular capsule 3) pericellular channels 4) territorial matrix 5) interterritorial matrix*

Outside the pericellular capsule the collagen-concentration is not the same everywhere. Direct around the capsule, the territorial matrix, the collagen concentration is lower than in the interterritorial matrix, which lies on a larger distance from the chondrocytes. The interterritorial matrix is a matrix of collagen fibres, as described in paragraph 3.2.2.

In the unloaded phase the collagen fibres in the interterritorial matrix lie in a waved pattern, but when they become loaded they immediately stretch in the direction perpendicular to the load-direction. As collagen only stretches when it is loaded, it is elastic and transformable. The collagen immediately relaxes, and comes back in the waved pattern, when it is unloaded. Noticeably the collagen fibres, which are located near the pericellular capsule, nr. 4, relax when the cartilage is loaded. So another load-distribution appears in the neighborhood of the chondrocytes.

3.3 Tissue engineered cartilage

Cultivation of tissue-engineered cartilage in vivo is subject of investigation. Cartilage tissue growth is a complex interplay between the cells, media, matrix components, mechanical stimulation and operation strategy. Several experimental studies have been done to describe parts of this interplay. The studies described below, give a good description of physical characteristics and measurement methods. The experiments are executed to understand the process and not with the intention to control the process. In the description of the experiments a clear and useful distinction is made between measured outputs and control inputs. The experiments described are examples of feedforward control, the whole traject is prescribed and meanwhile no intervention is executed. A clear distinction can be made between the experiments of Vunjak-Novakovic et al.(sections 3.3.1-3.3.5) and Bader et al (sections 3.3.6-3.3.6). The first examined the behaviour of (tissue-engineered) cartilage on the whole, while Bader examines the behaviour of seperated chondrocytes on mechanical loading. At the end of this section a method for producing tissue engineered cartilage is described.

3.3.1 Influence of cell seeding

Cell seeding of three-dimensional polymer scaffolds is the first step of the cultivation of engineered tissues in bioreactors. Seeding requirements of large scaffolds to make implants for potential clinical use include:

- high yield, to maximize the utilization of donor cells
- high kinetic rate, to minimize the time in suspension for anchorage-dependent and shear-sensitive cells
- high and spatially uniform distribution of attached cells, for rapid and uniform tissue regeneration.

This first step is investigated by [Vunjak-Novakovic et al., 1998]. Highly porous, fibrous polyglycolic acid (PLA) scaffolds, 5-10 mm in diameter and 2-5 mm thick, were seeded with bovine articular chondrocytes in well-mixed spinner flasks. Essentially, all cells attached throughout the scaffold volume within 1 day. Mixing promoted the formation of 20-32 mm diameter cell aggregated that enhanced the kinetics of cell attachment without compromising the uniformity of cell distribution. The kinetics and possible mechanisms of cell seeding were related to the formation of cell aggregates by a simple mathematical model that can be used to optimize seeding conditions for cartilage tissue engineering.

3.3.2 Quantitative analysis of glycosaminoglycan distribution

Analysis of the spatial distribution of glycosaminoglycans (GAG) in sections of cartilaginous tissues, engineered under different culture conditions could be used to correlate the effects of environmental factors with the structure of the regenerated tissue. [Martin et al.,1999] described a computer-based technique for quantitative analysis of safranin-O stained histological sections, using low magnification light microscopy images. In their investigation a parameter to quantify the intensity of red color in the sections was identified, which in turn was proportional to the biochemically determined wet weight fraction of GAG in corresponding tissue samples, and to describe the spatial distribution of GAG as a function of depth from the section edge. A broken line regression model was then used to determine the thickness of an external region, with lower GAG fractions, and the spatial rate of change in GAG content. The method was applied to the quantization of GAG distribution in samples of natural and engineered cartilage, cultured for 6 weeks in three different vessels: static flasks, mixed flasks and rotating bioreactors.

3.3.3 Influence of microgravity

While bioreactor research in outerspace is relatively new, it shows great promise. Mir Increment 3 (16 sept 1996-22 jan 1997) grew specimens of tissue engineered cartilage that surprised investigators. In [www.nasa] is

stated that Dr. Lisa E. Freed of MIT and her colleagues reported [Langer et al., 1997*] that initially disclike specimens tend to become spherical in space, demonstrating that tissues can grow and differentiate into distinct structures in microgravity. The Mir samples were smaller, more spherical, and mechanically weaker than Earth-grown control samples. These results demonstrate the feasibility of microgravity tissue engineering and may have implications for long human space voyages and for treating musculoskeletal disorders on earth. NASA has invented the rotating wall vessel bioreactor. It spins the fluid medium filled with cells to neutralize most of gravity's effects and encourage cells to grow in a natural manner. The rotating bioreactor was invented as a model of microgravity effects on cells. Ground tests of the bioreactor yielded three-dimensional tissue specimens approximating natural growth, a striking change from the pancake shapes of traditional cultures.

3.3.4 Influence of gas exchange

[Obradovic et al., 1999] have investigated the influence of gas exchange on the cultivation of tissue engineered cartilage. Cell metabolism and tissue composition were studied for engineered cartilage cultured for 5 weeks using bovine articular chondrocytes, polymer scaffolds and rotating bioreactors. Medium pH and concentrations of oxygen, carbon dioxide, glucose, lactate, ammonia, and glycosaminoglycan were varied by altering the exchange rates of gas and medium in the bioreactors. Cell-polymer constructs were assessed with respect to histomorphology, biochemical composition and metabolic activity. Low oxygen tension and low pH were associated with anaerobic cell metabolism, while higher oxygen tension and higher pH were associated with more aerobic cell metabolism. Under conditions of infrequent medium replacement, cells utilized more economic pathways such that glucose consumption and lactate production both decreased, cell metabolism remained relatively aerobic and the resulting constructs were cartilaginous. More aerobic conditions generally resulted in larger constructs containing higher amounts of cartilaginous tissue components, while anaerobic conditions suppressed chondrogenesis in 3D constructs.

3.3.5 Effect of mixing intensity

Mechanical forces regulate the structure and function of many tissues in vivo. [Gooch et al., 2001] investigated the effects of the hydrodynamic environment on tissue-engineered cartilage, by seeding primary bovine calf chondrocytes on fibrous polyglycolic acid (PLA) meshes and culturing in spinner flasks either statically or at one of nine different turbulent mixing intensities. In medium from unmixed flasks, CO_2 accumulated and O_2 was depleted, whereas in medium from mixed flasks the concentrations of both gases approached their equilibrium values. Relative to constructs exposed to non-mixed conditions, constructs exposed to mixing contained higher fractions of collagen, synthesized and released more GAG, but contained lower fractions of GAG. Across the wide range of mixing intensities investigated, the presence or absence of mixing, but not the intensity of the mixing, was the primary determinant of the GAGs and collagen content in the constructs. The all-or-none nature of these responses may provide insight into the mechanism(s) by which engineered cartilage perceives changes in its hydrodynamic environment and responds by modifying extracellular matrix production and release.

3.3.6 Influence of mechanical loading on isolated chondrocytes

Articular cartilage is subjected to dynamic compressive loading during normal activity. This loading influences chondrocyte metabolism through various mechanotransduction pathways. [Lee et al., 2000] investigated the influence of mechanical loading on isolated chondrocytes seeded in agarose constructs. The seeded chondrocytes were isolated from full depth cartilage or separately from the superficial and deep zone tissue. Beside the influences of mechanical loading the role of nitric oxide as a mediator of mechanical-induced effects has been studied.

Chondrocytes were isolated, separately, from full depth, superficial and deep zone cartilage and seeded in 3% agarose constructs. A cell straining apparatus was used to apply dynamic compressive strain to the constructs using a range of frequencies. Glycosaminoglycan synthesis, cell proliferation and nitrite production were assessed.
The studies in article [Lee et al., 2000] demonstrated that glycosaminoglycan synthesis and proliferation are influenced by dynamic strain regimes in a distinct manner. Indeed the data suggest that these processes occur in different chondrocyte sub-populations. It may be speculated that nitric oxide act as a mediator of mechanotransduction processes proliferation primary in the superficial cell sub-population.

3.3.7 Chondrocyte deformation at cellular and sub-cellular levels

Mechanotransduction events in articular cartilage may be resolved into extracellular components followed by intracellular signalling events, which lead to altered cell response. In [Lee et al., 19...] cell deformation is examined using a model involving bovine chondrocytes seeded in agarose constructs. Viable fluorescent labels and confocal laser scanning microscopy were used to examine cellular and sub-cellular morphology. It was observed that cell size increased up to day 6 in culture, associated with an increase in the contents of proteoglycan and collagen. The organization of the cytoskeleton components, revealed temporal changes. The constructs on day 1 were also subjected to unconfined compressive strains. A series of confocal scans through the centre of individual cells revealed a change from a spherical to an elliptical morphology. This was demonstrated by a change in diameter ratio. Using simple equations, volume and surface areas were also estimated from the scans. The volume does not change much with increasing construct strain, but the area appeared to increase significantly. Investigation with transmission electron microscopy revealed ultrastructural detail at the cell periphery, and suggests that this can be due to unraveling of folds at the cell membrane. Cell deformation was associated with a decrease in the nucleus diameter, in the direction of the applied strain. The resulting nuclear strain in one direction increased in constructs compressed at later time points, although its values at all three assessment times were less than the corresponding values of cell strain. It is suggested that the nuclear behaviour may be a direct result of temporal changes observed in the organization of the cytoskeleton.

3.3.8 Method for tissue engineering cartilage by seeding on bioresorbable scaffolds

[Schreiber et al., 1999] describe a method for the in vitro generation of a 3D cartilage matrix from articular chondrocytes seeded onto a bioresorbable polymeric scaffold. The tissue is cultured statically (in petri dishes). The tissue is cultured for 2 weeks or more. After culturing the construct between 4 and 6 weeks a homogenous tissue results containing up to 2% S-GAG, 0,9% total collagen, and 90% water of the total construct weight. These levels approach the lower levels of normal articular cartilage. This article [Schreiber et al., 1999] contains well described histologic, biochemical and molecular analyses, which offer useful information and references [Kim et al., 1998*] [Woessner, 1961*] about measurement methods.

Chapter 4

Control

4.1 Process description

To produce tissue-engineered cartilage, autologous chondrocytes are seeded onto a biomaterial scaffold. The scaffold offers the structure on which new tissue is formed. The scaffold material should posses mechanical integrity and be resorbable. The scaffold with chondrocytes is placed in a bulk fluid in a bioreactor, the IMBIOTOR. In this bioreactor, the chondrocytes produce a new ECM of collagen fibres and proteoglycans. The chondrocytes withdraw nutrients from the bulk fluid by diffusion, osmosis and perfusion to build new extracellular matrix (ECM). During this process toxic waste products are formed. These products need to be secreted by diffusion,osmosis and perfusion. Both exchange processes are slow transport processes.

The neo tissue growth is stimulated by applying mechanical load to the scaffold. This form of stimulation is mainly done to obtain comparable tissue structure as in vivo.

In order to minimize the presence of regions with necrotic cells, the nutrients and waste products need to move freely through the cellular construct. Inadequate tissue conditions are currently a major problem for processing constructs of greater than 2 mm in thickness. The tissue that will be produced in the IMBIOTOR is a device of 40x50 mm in area, with a thickness of 2-10 mm, ideally 4 mm.

Tissue processing requires the application of biochemical and biophysical stimuli for induction of appropriate and enhanced cellular growth, biosynthesis and tissue remodelling. Furthermore, differences in the inherent metabolic characteristic of cells isolated from different patients will lead to variability between end-point implants. So, the development of the mechanical integrity of the new tissue is influenced by external and internal factors. If the mechanical integrity could be measured, then figure 4.1 would visualize the development of the mechanical integrity of the new tissue and the scaffold material.

A certain level of mechanical integrity is needed before the tissue-engineered cartilage can be implemented in the human body. The mechanical integrity of the tissue-engineered cartilage is determined by the integrity of the neo-tissue and the scaffold material. As the development of both materials is influenced by external and internal factors, the development of mechanical integrity differs from case to case. Due to this uncertainty it is hard to establish the moment of implementation. This moment and the required properties of the tissue-engineered cartilage at this moment, still have to be determined in arrangement with the other partners of the IMBIOTOR-project.

In order to design a bioreactor it has to be taken into account that storage, handling and applications in a clinical environment poses significant hurdles, as live tissues cannot be easily shipped.

The development of a successful neo-cartilage tissue depends on a complex interplay between the cells, media, matrix components, design, mechanical stimulation and operation strategy. The optimized interplay will be investigated and will result in an intelligent mechanically stimulated bioreactor.

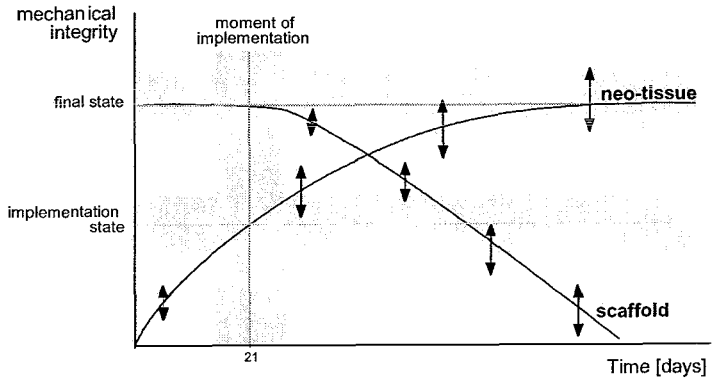

Figure 4.1: *If mechanical integrity could be measured then this figure would visualise the development of the neo- tissue. The moment of implementation of tissue-engineered cartilage depends on the mechanical integrity of the neo-tissue and the scaffold material. The development of both materials is a function of external and internal factors.*

4.2 Optimization criteria

In order to design a controller the aim of the optimization process has to be described in optimization criteria. These criteria describe why a control system is used. These criteria have to be translated in several variables which value has to be kept near some target value. The target value may be constant, in which case the objective is regulation, or it may vary, and the problem becomes one of tracking.
The aim of optimizing the production of tissue-engineered cartilage is:
Designing an intelligent bioreactor control system with operation strategies which minimize production time and maximize tissue function and cell viability. The created implant needs to have sufficient mechanical integrity to function in the human body.

4.3 System model

The production of neo-articular-cartilage tissue will be guided by a control system, in order to guarantee the control criteria are optimized. To understand the control system, the physical elements of a control system are introduced first and visualized in figure 4.2.

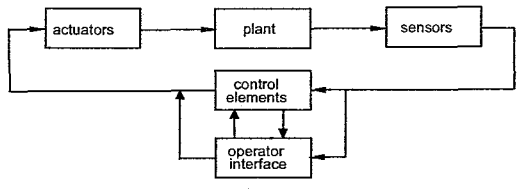

Figure 4.2: *Physical components of a control system*

The word plant is generally used to denote the object under control; in this context it is used for the production

process of neo-articular cartilage. A plant has output variables, some of which are the ones to be controlled; in this context the outputs are the characteristics of the neo-tissue and the characteristics of its environment. These variables can be measured by sensors. A sensor is basically a transducer, i.e., a device that transforms one type of physical quantity into another, usually electrical. A plant must have input variables, which can be manipulated to affect the outputs. The elements that permit these manipulations are called actuators. The role of control elements is to carry out the control strategy, i.e., to derive command signals for the actuators in response to the sensor outputs. The operator interface is the window to the outside world that allows human monitoring and intervention. It receives information concerning the input and outputs, plus certain status variables from the control elements. The process scheme used for the control of production of tissue engineered

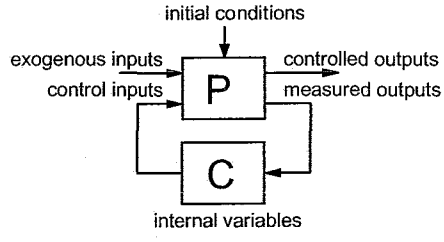

Figure 4.3: *Control scheme of the IMBIOTOR*

cartilage is shown in figure 4.3. The P represents the production process of tissue-engineered cartilage. In this process scheme two types of outputs are distincted, namely controlled outputs and measured outputs. The controlled outputs are the outputs which are desired to be controlled. They are derived from the optimization criteria. Some of them can be measured, and some may not. To fulfill the optimization criteria several plant variables are measured, known as measured outputs. The control inputs are the inputs that are manipulated during control to affect the output. The value of the manipulated inputs depends on the control strategy in combination with the value of the measured outputs and internal variables. Internal variables are variables which value is obtained from computations of the controller element. The strong inter-relation of variables makes controlling complex. The exogenous inputs consists of inputs which prescribe the reference trajectory and it also consists the undesired inputs known as disturbances. The system behaviour is determined by the initial conditions of the system. Some of those initial conditions are prescribed in order to obtain the right system behaviour.

4.4 Classification of signals

In the paragraphs below the inputs and outputs are defined and classified. Appendix A contains a short structured survey of all these inputs and outputs

4.4.1 Controlled outputs

The controlled outputs are derived from the optimization criteria. These outputs are desired to be controlled to obtain optimal production of tissue-engineered cartilage. With the information obtained with measurements the controlled outputs can be controlled.
The tissue function has to be optimized in order to assure the final implant has sufficient *mechanical integrity* to function in the human body. The micro and macro environment has to have acceptable biochemical and mechanical properties. Which will be 20 to 80 percent of real articular cartilage tissue. The real bounds still

have to be defined. This has to be done in arrangement with the other partners of the IMBIOTOR project and with more information of the product and process.
To develop and maintain a tissue that functions well the chondrocytes have to be *viable* and active, because they produce the new tissue. So the cell viability and the cellular activity are controlled outputs. Waste accumulation of toxic products have to be taken care off, because a too high concentration harms the chondrocytes. To guarantee the tissue stays alive in the human body sterility has to be guaranteed. The latter is more a constraint than a controlled output
The *production time* is the time from placing the scaffold with chondrocytes in the bioreactor till the time the neo-tissue is ready for implementing in the human body. The production time depends on the development of the mechanical integrity of the neo tissue and the mechanical integrity of the scaffold material. So optimized tissue growth, tissue function (macro and micro environment), cell viability and optimization of the nutrient exchange process is demanded.

4.4.2 Measured outputs

Several outputs will be measured to register and optimize the development of neo-tissue formation.
Two kind of measurements can be distinguished, namely on-line and off-line. In the on-line case the output can be monitored during the production process and can immediately be used for control. Examples of on-line measurement methods are optode sensor technologies and low cost needle-type sterilized enzyme electrodes for glucose, lactate and pyruvate.
Two types of off-line measurements can be used:

- Whole or partial sampling of the construct. This involves removing all or part of the construct, for subsequent analysis and can not be performed in real time. The analysis data can be used for control. For example measuring cell mass production.
- Sampling of the fluidmedium so the construct can be left in place and intact. The relationship have to be found between elements present in the medium and in the construct itself. This measurement method requires a complex data analysis.

Some measurement methods are not useful for monitoring the development of the tissue, because of their destructive character. Examples tissueof thereue to these measurement methods the created tissue can not be used as an implant anymore. The positive effect of these measurement methods is that they offer information that can be used for the development of a process or control model. So before developing the real control model a research control model will be developed which uses these methods.
Not all controlled outputs can be measured, but all controlled outputs can be characterized by measurable outputs. The measurable outputs are listed below and are grouped per controlled output. If the measurement technique of the measured output is known, then it is stated behind the output. The list below is preliminary and will change when more is known about the measurement methods. Not all measurement methods are known yet, further investigation in the sensor/measurement technology is needed too.

- *Cell viability:* cell viability (fluorometric), cell number (fluorometric DNA dye binding assey), dead biomass concentration (sample), cell size (optode sensors)
- *Macro environment:*

Biochemical: extracellular matrix synthesis (optode), composition of the bulk fluid (samples), proteoglycan concentration, GAG concentration (fluorometric), pH (sample), O_2 concentration (sample), streaming potential, construct weight ->wet weight/dry weight.
Mechanical: hydrostatic pressure, flow rate, strength, pressure, stiffness
Morphological: ECM organization (optode), porosity (), cell biomass concentration, collagen density,

medium flow rate, type of collagen I or II (specific antibodies), tissue fixation and embedding (sample), total collagen (hydroxyproline content from acid-hydrolyzed)

- *Micro environment:*

Chemical: enzymes, matrix metalloproteinases, streaming potential, non-invasively pO_2 and pH (fluorometric based methods)
Mechanical: local stiffness, local strain magnitude, nucleus deformation
Genetic: mRNA

- *Cellular activity:* extracellular matrix synthesis, cell number (optode), biomass concentration (sample), cell differentiation, cell death, medium temperature (sample)
- *Waste accumulation:* concentration of (toxic) waste products, NO concentration
- *Nutrient exchange process:* nutrients concentration (intra/extracellular), composition of the bulk fluid, osmotic pressure
- *Time:* time
- *Sterility:* interleukins, antigens, viruses, tumour cells, foreign constituents

4.4.3 Internal variables

Some characteristics of the production of tissue-engineered cartilage can not be measured, but they can be computed out of measurement data with the plant model or control model. Internal variables are variables which are computed by the controller to characterize the system. They give a better view of the state of development of the tissue. The outputs of the harmful measurement methods, mentioned in the previous paragraph, can be used for the development of the plant and control model. In the real model these outputs are not measured and thus can be seen as internal variables. Other examples of internal variables are the neo-tissue production rate and cell growth rate. The number of internal variables will further increase when the exact measurement methods are known.

4.4.4 Initial conditions

Before starting the production of tissue-engineered cartilage the initial conditions has to be determined. These conditions partly determine the system behaviour. Some conditions are prescribed, while others are given. These initial conditions are for instance dimensions of the scaffold, concentration of seeded cells, bulk temperature, sterility of the bioreactor, initial medium composition. The number of initial conditions will increase when the process in better understood, and the controller is further developed.

4.4.5 Exogenous inputs

The exogenous inputs contain two parts: a reference trajectory and disturbances.
A reference trajectory is a predefined development traject that has to be followed to optimal produce tissue-engineered cartilage. So the development from scaffold with seeded cells to implant has to be prescribed, this is done by prescribing the culture conditions in time. Examples of them are: time, medium temperature, adding of stimuli, refreshing of fluid-medium.
Disturbances are undesired inputs. They influence the system behaviour like the other inputs do. Their effect has to be minimized by the control system. Examples of disturbances are: environmental temperature, environmental humidity, sterility of the environment

4.4.6 Control inputs

Control inputs are inputs that can be manipulated during control to effect the outputs. The aim of controlling is that the controlled outputs exactly follow the prescibed trajectory. The control inputs are altered with the information of measured outputs and internal variables. The control inputs can be categorized by chemical input factors and mechanical input factors. Chemical input factors are: adding of nutrients, refreshing the medium, altering the composition of the medium. The mechanical input factors are: magnitude of the load, magnitude of stress, aeration rate.

4.5 Comparable process control systems

The great power of control theory is that it is applicable for many types of systems. The power of control theory is derived from its ability to fit a multitude of different problems into a single abstract, mathematical framework. So it is useful to look for comparable process control systems. These control systems may offer mathematical frameworks, which may be useful for the development of the framework of the IMBIOTOR. In this section several models, estimators and controllers are described.

4.5.1 Animal cell culture

A key question in (bio)process control is how to monitor reactant and product concentrations and product parameters like reaction rates in a reliable and cost effective manner. However, it appears that in many practical applications, only some of the concentrations of the components involved that are critical for quality control are available for on-line measurement. For instance, dissolved oxygen concentration and gaseous flow rates are available for on-line measurement while the concentration values of biomass, substrates and/or synthesis products are often available via off-line analysis. In [Perrier et al., 1998] an interesting alternative is described: Luenberger or Kalman observers. They exploit the use of a model in conjunction with a limited set of measurements. In these techniques, a model, which includes states that are measured as well as states that are not measured, is used in parallel with the process and the model states may then be used for feedback. This configuration may be used to reduce the effect of noise on measurements as well as to reconstruct the states that are not measured. These concepts were originally developed for linear problems. The concepts have been extended and exploited, that it can be used for nonlinear characteristics of bioprocess dynamics. Linearized versions (the linearized tangent model) of the process dynamics are computed from a Taylor's series expansion of a state space model around some equilibrium point, and the observer theory referred to above can be applied. Modified observers, particularly the extended Kalman filter, have found applications in some (bio)chemical processes. One of the reasons of the popularity of the extended Kalman filter is that it is easy to implement since the algorithm can be derived from the state space model. In [Perrier et al., 1998] several minor points of this methods are mentioned:

- The stability and convergence properties are essentially local and valid around a equilibrium point
- It is rather difficult to guarantee its stability over wide ranges of operations
- Ljung (1979) showed that the extended Kalman filter for state and parameter estimation of linear systems may give biased estimates or even diverge if it is not carefully initialized.
- The theory for the extended Luenberger and Kalman observers is developed using a perfect knowledge of the system parameters, in particular the process kinetics: it is difficult to develop error bounds and there is often a large uncertainty on these parameters.

The design of monitoring tools for the on-line estimation of process variables and parameters has been quite an active research area. Alternative approaches have been proposed to the extended Kalman filter that uses

process physics in a more direct manner to develop nonlinear observers applicable to the estimation problem of (bio)chemical reactors. The proposed observers are based on the well-known nonlinear model of the process without the knowledge of the process kinetics being necessary. For state estimation these observers have been called asymptotic observers; for parameter estimation, observer-based estimators have been developed. The development of new state and parameter estimators is a very active research area. Other options are network based estimators [Cannon and Slotine, 1995*] and H_∞ based estimators [Reza Meheimani et al., 1996*]. Compared to these approaches, the observer-based estimator presents the following specific characteristics: it does not need to introduce a black-box (nonlinear) modelling of the uncertain parameters, and the stability analysis does not lead to conservative tuning rules (as may be the case with H_∞ based algorithms).
Initially observer-based estimators were designed for estimation on-line kinetic parameters, like specific growth rates, in bioprocesses, and have proved to be very successful in practical applications. In article [Perrier et al., 1998] a systematic tuning approach, of the observer-based estimators, that allows a decoupled estimation of each parameter and the assignment of the estimator dynamics independently of the process dynamics is described. The presented approach is illustrated on an animal cell culture example in numerical simulation and with real-life data.

4.5.2 Bioremediation processes

In situ bioremediation is a technology for cleaning up subsurface contaminated with hazardous materials. Bacteria are the key players in bioremediation. Micro organisms posses enzymes, which use environmental contaminants as food and enable the breakdown of contaminants into less harmful substances. Organic contaminants undergo microbial transformation because they (the contaminants) are a source of carbon, the basic building blocks for new cells, and electrons. The transfer of electrons from an electron donor (usually an organic compound) to an electron acceptor (usually oxygen) is accompanied by a release of energy which is used by organisms to multiply (metabolism).
Examples of applications for bioremediation are restoring polluted groundwater to conditions fit for drinking and removing air from volatile organic compounds.
In situ bioremediation is a remediation technology in which the indigenous subsurface bacteria are stimulated by injecting compounds to provide food and energy. The way the compounds are injected is a crucial component of the technology. [Chawla et al., 2000] use techniques from the theory of optimal control of distributed parameter systems [Lenhart and Protopopescu, 1994*] [Lions, 1961*] [Lions, 1971*] to characterize an 'optimal ' injection function in a tube bioreactor. The state system, the set of equations that govern the evolution of bioremediation, is a 'hybrid ' system consisting both partial and ordinary differential equations. S.Lenhart et al. prove the existence and uniqueness of solutions of the state system and they prove the existence of optimal control. The optimal control is characterized in terms of the optimality system, which consists of the state system and a suitably chosen adjoint system. The uniqueness of the optimality system is proven for "small"time. Finally an explicit finite difference scheme is used to discretize the optimality system coupled with an iteration scheme to get a concrete profile of the optimal injection function.
In [Handagama and Lenhart, 1998] another application for optimal control of a PDE/ODE system is described, namely modelling a gas-phase bioreactor.

4.5.3 Fermentation processes

Bacterial fermentation

In controlling a fermentation process the most difficult problem is to determine the current state. This problem can be overcome if a suitable process model can be found, because then an estimator can be used to determine the non-measurable variables. In [Jalel et al.,1993] alternative approaches for modelling and state estimation of a typical, industrial fermentation process are described. First an autoregressive technique is adopted to develop a model using process data from the full scale production plant. This identification technique is

a linear time invariant approach while fermentation is a highly non-linear time varying process. Therefor different techniques are investigated to create a general time varying model to represent the process over the whole operating range. These techniques are sequential modelling, time-varying model and difference equation model. A multi layer neural network has been developed to model the state variables of the process. Inputs of the network are the on-line measurements, while the outputs are the off-line measurements whose values are to be estimated. In the fermentation process one approach to control is to define a desired trajectory for the process and then to drive the process along the trajectory by controlling the amounts of nutrient feed. Controlling the states of the process around a defined trajectory is achieved using two techniques: self organizing fuzzy logic controller and a model reference variable structure controller.
[Rivera and Karim, 1993] describe a hybrid neural network-genetic algorithm for bioprocess optimization, see figure 4.4. A recurrent neural network is supervised-trained on a set of available fermentation data, and used to predict the species concentrations which are difficult to measure on-line.

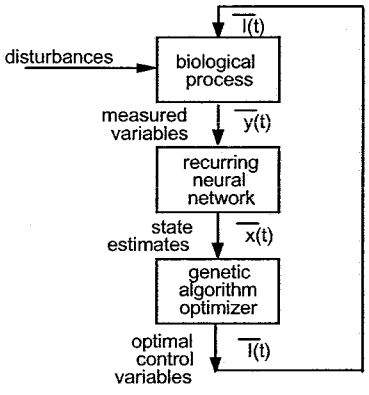

Figure 4.4: *Blockdiagram of the hybrid optimization scheme*

With the state variable information available, a micro-genetic algorithm is used to generate the optimum control settings to improve the process performance. The hybrid optimization technique was applied to the fermentative ethanol production by Zymomonas mobilis in batch mode. The optimization problem can be expressed as finding the best environmental conditions to maximize the product yield.

Bakers' yeast fermentation

In [Yuan and Bellgardt, 1993] three experiments of quality control were described. In these experiments the storage stability for compressed baker's yeast was optimally controlled by means of minimizing the fraction of budding cells in cell population. The time series for the optimal feeding rate was obtained by two models: a metabolic [Yuan et al., 1991*] [Yuan et al., 1993*] and a cell cyclic model [Bellgardt, 1983*] [Bellgardt and Yuan, 1991*].
Bioprocesses are usually highly nonlinear with dynamics that vary in time. For this type of processes adaptive optimization techniques with on-line system identification can be very attractive. Several methods have been designed, among them:

- On-line optimization by linear dynamic model identification based on least squares and instrumental variable techniques [Rolf and Lim, 1984*].
- Adaptive optimization scheme which does not require the use of gradient information from a linear model [Harmon et al., 1987*]

- Well-behaved approximate nonlinear model which represents steady-state behaviour by incorporating a priori knowledge of the system [Golden and Ydstie, 1989*].
- Multivariable adaptive optimization of continuous bakers' yeast fermentation by the control of the dilution rate and temperature [Chang and Lim, 1989*].

However, all these methods are based on a linear dynamic model or an approximate nonlinear model which is limited for a small range of operating conditions. In article [Chen and Weigand, 1992] a novel approach to applying an on-line optimizing control strategy to a continuous fermentation process is described. A recursive backpropagation neural network identification algorithm with forgetting factor is described for the on-line modelling of the process. Also, an on-line adaptive optimization technique which uses a gradient method based on the identified dynamic neural network is proposed. The general structure of this adaptive optimization technique is shown in figure. The adaptive optimization technique was applied on bakers' yeast fermentation process. The advantages of this method are its applicability to other processes and that no kinetic model is needed for this RBPN optimization approach, the RBPN model can be developed directly from process data.

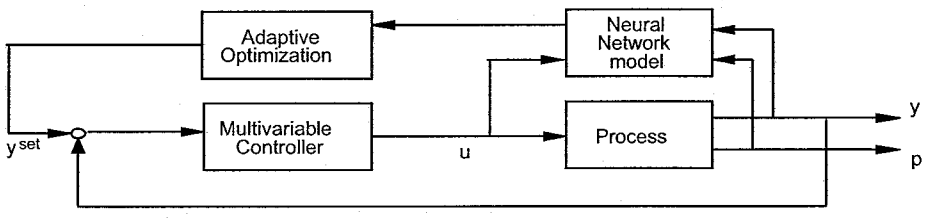

Figure 4.5: *General structure of adaptive optimization based neural network model*

Feedback control requires measured values of variables that need to be regulated. The benefits of feedback control, continuous monitoring and optimization are difficult to realize in a bio-process, due to the lack of regular on-line measurements of key variables. Further, the process has characteristics that vary in time in a non-linear way. Thus a fixed linear model cannot be used in the model-based predictive control algorithms. In a bio-process the states of secondary importance such as carbon dioxide and oxygen contents in exit gas are measurable at a fairly rapid and regular rate. Multi-rate system identification makes it possible to generate optimal estimates of the primary variables at minor sampling instants from secondary innovations. In [Gudi and Shah, 1993] a novel approach of adaptive multi-rate filtering and estimation is presented. To generate optimally filtered state estimates conditioned on the best available model when the measurements arrive at different sampling rates, model based multi-rate Kalman filtering (MKF) technique of Glasson [Glasson, 1980*][Glasson, 1983*] is used. The model that is needed for the MKF based filtering, is adapted to the time varying characteristics of the process using the maximum likelihood based sequential parameter updating strategy of Ljung and Soderstrom [Ljung and Soderstrom*]. The multi-rate adaptive estimator thus utilizes information in both the primary and secondary measurements in an optimal way to generate regular, filtered estimates of all the states from frequent measurements of the secondary states and infrequent measurements of the primary states. The strategy is experimentally evaluated by application to a fed-batch bioreactor for bakers' yeast.

Continuous stirred tank fermenter

Many chemical and agricultural processes, such as exothermic processes and biological reactors, often operate around unstable steady states. To approximate their dynamics for the purpose of controller design, unstable

first order dead-time (UFOPDT) transfer function models are used. In [Arvanitis et al., 2000] several new methods, based on a variety of set-point filters and on some new accurate approximations of the crossover frequency, for tuning PID controllers for these UFOPDT processes are reported. The proposed methods require small computation rates and they are particularly useful for on-line tuning. The methods are demonstrated on a continuous stirred tank fermenter.

Solid substrate processes

In article [Fernandez et al., 1997] a preliminary control system for a solid substrate pilot bioreactor is described. The control structure and control logic is described, i.e. the available variables for monitoring and manipulation, the pairing among them and the control algorithms used. The available variables are listed in tables with their type of measurement (on-line or off-line) and the measurement instrument. This set up for controller design is very practical and can be used as an example for the controller design for the IMBIOTOR.

4.5.4 Waste water processes

Waste water processes are an active research area in the control community. [Carlsson et al., 1993] describe the on-line identification of the oxygen transfer rate, K_La and the respiration rate in an activated sludge process. The respiration rate has normally a diurnal variation and the oxygen transfer rate is nonlinear. The nonlinear K_La function is modelled with a static, constrained piecewise linear model, derived from the mass balance of the dissolved oxygen, while the respiration is modelled as a random walk. A Kalman filter type recursive identification algorithm is applied in order to estimate the oxygen transfer rate and the respiration rate from measurements of dissolved oxygen concentration and air flow rate. An important feature of this identification procedure is that a separation of the a priori known rates of variation of the individual parameters can be utilized to improve the estimates. The approach is illustrated with simulated and real data.
The primary objective of the newer waste water treatment processes designed for nutrient as well as organic removal is multivariable: effluent quality criteria is to be maintained with respect to organic, nitrogen and phosphorus contents. Proper control of such processes is difficulted, by the lack of effective variables which can be manipulated. [Isaccs et al., 1993] examined a new control variable, namely the addition of an external carbon source to the point in the process at which denitrification occurs. The used control strategy is basically feedforward in nature. Once a decision is made to add chemical oxygen demand (COD), this is done at a constant rate. The only feedback element involved is a decision to terminate addition when the measured concentration drops to zero. An adaptive algorithm based on an empirical model describing the relation between denitrification rate and COD addition rate serves as a means to adjust the amount of COD addition to meet the current demands.
The system described in [Nihtila et al., 1996] is a continuous-flow fixed bed bioreactor. It is aimed at removing harmful nitrogen (and carbon) compounds from the influent waste water. The goal is to decrease the overall concentration at the outlet of the reactor under some prescribed allowable limits. The process is modelled by a set of two partial differential equations of parabolic type. The model is nonlinear due to the dependence of the specific growth rate of the microorganisms on the concentrations of nitrogen and biomass. The model has two independent variables: the time-to-go and the space variable, the distance from the inlet of the reactor. The key technique in studying the behaviour of and designing a controller for the system is to approximate the infinite-dimensional PDE model by a nonlinear finite-dimensional ordinary differential equation (ODE) model. A method of orthogonal collocation [Tali-Maamar, 1993*] and a finite element method (FEM) of Galerkin type [Nihtilä, 1994*] have been applied to construct the approximations. For some parameter values of the PDE model the linearizing control of the semidiscretized model results in unstable behaviour in the sense that the zero dynamics of the model is unstable. This same unstable behaviour was also earlier observed in using the orthogonal collocation for the semidiscretization. The connections between the location of the zeros of the original model PDE model linearized around its steady-state solution and the stability/instability properties

of the linearizing control of the semidiscrete model are discussed.

4.5.5 Plant cells growth processes

Micropropagation technology is currently applied to a large number of agricultural and forestry plant species. However, the high cost of in vitro propagation still limits the world wide economic expansion of this industry. Automation and scaled-up liquid cultures for in vitro plant propagation are mandatory to overcome some of the limitations imposed by labor intensive and high production costs of existing conventional techniques. Bioreactors are used in micropropagation for both embryogenic and organogenic regeneration pathways. However, regulatory systems for the control of the internal environment and for defining the requirements for quality plant regeneration in bioreactors are still limited to a few crop species. In [Ziv, 1995] various factors are described that are related to the plant growth and the morphogenesis in liquid cultures. These factors are: method of aeration and circulation, effects of agitation, shearing forces, gaseous environment, pH, light, temperature, medium components, rheology and osmoticum and the level of growth promoting and growth inhibiting regulators. The factors described are inputs and outputs of the plant growth process to be controlled. In this article no controller is discussed at all, but this article offers understanding in the physiology of the growing (plant) biomass in bioreactors.

For designing a controller a model of the plant is needed. The plant cell growth process is a comparable process to the production of tissue-engineered cartilage. So, the models of plant cell growth may be useful. Several models are developed to improve the understanding of the growth phase by their ability to describe the behaviour of the bioprocess. This ability is directly related to the structure of the model and its kinetics forms. Many models have been developed to describe and predict the behaviour of plant cell cultures under different conditions. These models can be broadly classified into two categories: segregated and non-segregated models. Published models of plant cell cultures usually include the growth and production phases of the bioprocess.

Selection of growth variables for non-segregated models is generally based on nutrient limitation [Curtis et al., 1991*] [Gardiola et al., 1995*] [Pazoutova et al., 1981*] [Gulik et al., 1992*], combined with the Fencl logistic equation [Fencl, 1996*] [Quinlan, 1986*] [Taticek, 1990*], or associated with a leak from the cell to the medium of this nutrient [Frazier, 1989*]. As a result, the model describes the growth kinetics as a function of a specific nutrient while the remaining nutrients are assumed to be non-limiting. This type of model can be used to study the effect of a specific nutrient on the growth process but can not generally predict the whole growth phase adequately. Several segregated models have been presented: a segregated model that predicted the concentration of viable, non-viable and total biomass [Bailey and Nicholson, 1989*], previous model included with the description of nutrient uptake and the internal use with changes in biomass composition [Hooker and Lee, 1992*] [Gulik et al., 1993*], growth of four types of biomass together with the production of secondary metabolites [Grm and Mele, 1980*]. [Siroïs et al., 2000] presents a two-step approach which was used to develop a segregated model for the growth of *Eschscholtzia California* system which employs a novel aeration and agitation system, designed to enhance gaseous exchange and reduce shear stresses on submerged cell suspension cultures. In the first step, a non-segregated model was developed. Its parameters were estimated using least-square methods. The exponential phase of biomass growth was well predicted. On the other hand the model was unable to predict the decline phase. In the second step, a segregated model was developed to improve the understanding of the biomass state in a bioreactor. The biomass was divided into three components based on the hypothesis of different activities: small cell type a biomass, large cell type b biomass and large inactive cell type c biomass. To represent cell death, a mortality constant was associate with each type of biomass in the equation system. The estimation of total biomass growth was improved over the non-segregated model since the former model predicted better all growth phases.

4.5.6 Cultured skin grafts

In [Prenosil and Kino-Oka, 2000] an automated membrane bioreactor, named KERATOR, is described which was developed to produce Autologous Wound Dressing at significantly reduced cost and time of transplantation down to two weeks. A microscopic video system with image analysis was developed for on-line monitoring of the cell growth and morphology in the KERATOR. The computer uses the obtained information to control medium change and to predict the end of cultivation.
For image analysis a phase contrast microscope with CCD camera was used. The original image was captured with video digitizer card in the computer. The captured area of the original image was 0.83 mm^2. The value of confluences at several positions were determined by using the LabVIEW software with the add-on image processing software IMAQ Vision. [Prenosil and Kino-Oka, 2000]. The control strategy consists of a prescribed trajectory and feedback-mechanisms. It's a real controller, but the control algorithms are not mentioned. Wether the method of image analysis is also useful for the IMBIOTOR is not clear yet. The description of the method was too sparse, to conclude so.

4.5.7 WAU control processes

The systems and control group [www.wau1] of the Wageningen University of agrotechnology and food sciences (WAU) of prof. G.v.Straten has several research projects, which are related to the IMBIOTOR project. R. Neeleman is working on a PhD-project about vaccine production throughput, yield and quality could be enhanced by optimization of the batch process paths. The main objectives are the development of model-based sensors for vaccine production systems, the optimization of control strategies and the implementation of the methods in a pilot-plant.
The Food and bioprocess engineering group [www.wau2] of the Wageningen University of agrotechnology and food sciences of prof. H.Tramper and prof. R.Boom focuses on several research themes, under which Animal cell technology and solid-state fermentation. These research themes may offer a contribution to the controller design for the IMBIOTOR, so further research to this area is recommended.

4.5.8 Discussion

Controlling biological systems is dealing with systems which are characterized by uncertainties and poor data, time variability, adaptation and lack of standard models. In controlling the most difficult problem is to determine the current state of the process. This problem is overcome once a suitable process model is found as well as an adequate estimator for determining non-measurable variables. In the sections above several models, estimator and controllers are proposed. It is clear that finding a suitable model is not easy. Bioprocesses are complex processes to describe.
Linear model are easy to control and physically understand, but they can only be used locally and system knowledge is required. Nonlinear models are not that easy to control and still system knowledge has to be known. Neural networks offer a solution, because no system knowledge has to be available. The disadvantage of these systems is that they do not offer any physical understanding of the system.
After evaluation of several possibilities it is obvious that there is still no universal best method for modelling bioprocesses. Because the complexity of bioprocesses the use of artificial intelligence, i.e. neural networks and genetic algorithms, could be considered.
The non-measurable states of a process can be estimated by optimal estimators or by genetic algorithms. An optimal estimator is a computational algorithm that processes measurements to deduce a minimum error estimate of the state of a system by utilizing: knowledge of system and measurements dynamics, assumed statistics of system noises and measurement errors, and initial condition information. The advantages of this type of data processing are that it minimizes the estimation error in a well defined statistical sense and that it utilizes all measurement data plus prior knowledge about the system. The potential disadvantages are its sensitivity to errors in the a priori models and statistics. The controllers used to control the processes of above

are feedforward and (state)feedback controllers. They are not described in detail. The emphasis of the articles is on modelling and state estimation.

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

This report contains a control oriented investigation for tissue engineering of cartilage.
A first literature investigation in the product and the process offered insight in the production process and in the way the specialists handle it. The specialists are occupied in their specialistic area and there is no overall view of the process, which is necessary for modelling and controlling it. So, the task of the model and control designer is to obtain this overall view. This report contains lists of inputs and outputs which are a first step in acquiring this overall view.
Nowadays the implants are produced by recipes and are based on experiences of the past, which comes down to feed-forward control combined with simple feedback controllers to keep several process parameters constant.

The process in a bioreactor can be compared to other processes, for example chemical processes. Control design is an already much investigated topic in the chemical industry. For slow chemical processes model based control is often used. A model based controller tries to follow a desired trajectory. The control inputs are determined by using knowledge of the model. This control method offers the possibility to take constraints into account. By trying to develop a model based control system interaction between the specialists is stimulated. This interaction may result in a better understanding of the process. By designing a model based controller the designed model is verified with reality.
The European project is in its first phase and the workpackages in the project are executed parallel instead of sequential. So there is still no model is available, which makes it difficult to design a model based controller.

In order to explore other possible controllers literature research is done for several comparable processes. This investigation showed that bioprocesses are complex processes to model and to control, due to their non-linear behaviour, multiple in- and outputs, and non-measurable outputs. In literature several methods are described to model and control comparable processes. It has to be stated that in these articles the application of the methods are well described, but these articles do not offer a comparison with other methods. Concluding the results found in literature there is no universal best method for modelling and controlling bioprocesses. The investigated literature emphasizes process modelling, and process controlling is only slightly described. The applied controllers are simple controllers.The models and controllers in literature are based on measurement data.

In order to develop a model and a control system it will be necessary to obtain process data.

5.2 Recommendations

This report contains the first investigations for designing a control system for the optimal production of tissue-engineered cartilage. Much research is needed before the actual control system can be designed. A summary of the main issues is given below:

- An experimental set-up is necessary to obtain data for developing a model and controller.
- Consulting other partners of the IMBIOTOR project about the bioreactor concept is necessary to get interactions of different disciplines.
- A task of the control designer is making requirements for the model makers
- The inputs and outputs have to be refined with help of other partners of the IMBIOTOR project.
- Further investigation of control schemes is needed.
- Looking for available patents may give a better view of the current developments in bioreactor design.

Appendix A

Survey of inputs and outputs

This appendix contains a structured survey of inputs, outputs and internal variables of the primary controller design.

controlled output	measured outputs
cell viability	cell viability (fluorometric), cell number (fluorometric), dead biomass concentration (sample), cell size (optode sensors)
macro environment	
- biochemical	extracellular matrix synthesis (optode), composition of bulk fluid (sample), proteoglycan concentration, GAG concentration (fluorescence), pH (sample), O_2 concentration (sample),streaming potential, construct weight (wet weight/ dry weight)(sample)
- mechanical	hydrostatic pressure, flow rate, strength, partial pressure, stiffness
- morphological	ECM organization (optode), porosity, cell biomass concentration, collagen density, medium flow rate, type of collagen I or II (specific antibodies), tissue fixation and embedding (sample), total collagen amount (hydroxyproline content from acid-hydrolyzed)
micro environment	
- chemical	enzymes, matrix metalloproteinases, streaming potential, non-invasively pO_2 and pH (fluorescently based methods)
- mechanical	local stifness, local strain magnitude, nucleas deformation
- genetic	mRNA
cellular activity	ECM synthesis, cell number (optode), biomass concentration (sample), cell differentation, number of necrotic cells, medium temperature (sample), local temperature
waste accumulation	concentration of toxic waste products, NO concentration
nutrient exchange	nutrient concentration (intra/extra) cellular, composition of bulk fluid, osmotic pressure
time	time
sterility	interleukins, antigens, viruses, tumour cells, foreign constituents

Internal variables
neo-tissue production rate
cell growth rate

Initialconditions
dimensions of the scaffold
concentration of seeded cells
bulk temperature
sterility of the bioreactor
initial medium composition

Exogenous inputs	
reference trajectory	**disturbances**
aeration rate	sterility of the environment
refreshing of fluid-medium	environmental temperature
medium temperature	environmental humidity
adding of stimuli	

Control inputs
applied load
shear stress
loading orientation
adding of nutrients
adding of growth-factor
composition of medium
aeration rate
changing medium

Appendix B

Glossary in Dutch

agar: een gelatine achtig prodrukt dat wordt afscheiden door rode algen.

agarose: een suiker in zeealgen, dat agar de gelachtige structuur geeft.

allogenic cell: cell met hetzelfde celtype, maar met een andere genetische afkomst.

amino acid: amino zuur

anaeroob: zonder zuurstof plaatsvindend of kunnende leven

autologous cell: cell met hetzelfde celtype en met dezelfde genetische afkomst.

articular cartilage: gewrichtskraakbeen

bovine: runderachtig

calcified: verkalkt

cartilage: kraakbeen

chondrocytes: chondrocyten, de cellen in kraakbeen

collagen: collageen, lijmvormende eiwitstof die een hoofdbestanddeel van bindweefsel is

connective tissue: bindweefsel

cruciate ligament: gewrichts kruisband

cytoplasma: plasma buiten de celkern

cytoskeleton: systeem van eiwit filamenten in het cytoplasma van een cel. het levert de cel stevigheid.en de mogelijkheid tot gerichte bewegingen.

endoplasmatic retilicum: een membraan gebonden compartiment in het cytoplasma van cellen, waar vetten worden gesynthetiseerd en membraan-gebonden eiwitten worden gemaakt.

extracellular matrix (ECM): extra cellulaire matrix, met in kraakbeen als voornaamste bestanddelen: proteoglycans, collagen en water

golgi complex: een complex van cytoplasma organellen, betrokken met terminal glycosylation, membraan stroming, afscheiding, en afgifte van cellulaire producten naar buiten of binnen de cel.

glycol: glycolzuur

glycosaminoglycan (GAG): bouwsteen van proteoglycan. Bestaande uit herhalende dissacharide eenheden

graft: transplantaat

hyalene cartilage: type kraakbeen

in vivo: in het levende organisme

in vitro: in een kunstmatige omgeving

lactate: zout of ester van melkzuur

lacuna: holte

larynx: strottenhoofd

lymphatic vessels: lymfe vaten

mechanotransduction: .....

morphology: leer van vorm en bouw van de organismen

necrotic: afgestorven

osteoarthritis: chronische ontsteking van bot en kraakbeen

organel: een celbestanddeel die een specifieke taak uitvoerd, afgebakend door een omliggende membraan

protein: eiwit

proteoglycan: een van de hoofdbestandelen van ECM. deze moleculen bestaande uit eeen proteine kern, waaraan minstens een glycoaminoglycan covalent is gebonden

tissue: weefsel

scaffold: dragermateriaal

sternum: borstbeen

synovia: gewrichtsvocht

tendon: achillespees

trachea: luchtpijp

vascular: met bloedvaten

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