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<i>Abstract</i>	This document provides the requirements of the BIOTEX project. For each selected applications, it starts from the state-of-the-art in today's practice and technologies, provides the requirements for the respective applications from the user point-of-view as well as other stakeholders. The signals to be sensed and the corresponding sensors that are foreseen to address the requirements are listed and preliminary specifications are derived also for the on-body electronics.
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1. Introduction

“Prevention is better than cure”: this short statement is the essence of the BIOTEX project. The project’s aim is to supply textiles which do more than dressing: the project will provide a warning at the raise of some pathologic states using non-invasive monitoring techniques embedded into textile. The selected applications are:

- **Obese Children/sportive people:** it is very important for athletes to have a sufficient quantity of minerals and also avoid dehydration.
- **Diabetes:** an innovative textile will prevent the different pathologic states of patients with diabetes, which is of concern for many people.
- **Wound healing/skin graft:** the evolution of wounds will be tracked to avoid the appearance of a chronic wound or an infection.

A fourth application is considered for back-up reason: the evolution of implants will be monitored to early detect a possible implant rejection.

Several challenges will be faced during the project and it will probably not be possible to address all of them successfully in the frame of the project. For instance about sweat:

- The body fluid which is available (non-invasively) on the surface of the skin is sweat; little studies on sweat have been made possibly due to the difficulty to collect this fluid.
- Some sensors have never been used for sweat analysis yet; the techniques have thus to be validated first. More accurate sensor values will be available after trials with these sensors.

Section 2 of the document describes the fluids to be investigated and the parameters that make them. Section 3 describes the sensors which can be potentially used and which are planned for the project. The description of the selected applications and the respective requirements are provided in section 4. Section 5 summarises quality assurance and regulations aspects which are shared by all applications. A list of references is provided at the end of the document in section 6.

All references in this document to masculine conjugations (e.g. "he" and "his") are applicable to female as well as male persons.

1.1 Glossary, acronyms and abbreviations

Item	Description
ABI	Ankle-Brachial Index
ADC	Analogue-to-digital conversion (or converter)
Board	Project Management Team
BIOTEX	Bio-sensing textile for health management IST-2004-016789
BMI	Body Mass Index, i.e. the ratio of the body weight in kilograms and the square of the person height in meters
BPO	BIOTEX Project office – located at CSEM.SA – Neuchâtel – Switzerland
BPW	BIOTEX Project Workspace (Groove) only for BIOTEX project partners
BSA	Body Surface Area
CA	BIOTEX Consortium Agreement
CAS	Clinical Application Specialist
CFU	Colony-Forming Unit
Consortium	Group of all partners gathered in the BIOTEX Project
CPE	Conducting Polymer of Electricity
DEL	Deliverable
DKA	Diabetic Ketoacidosis
DoW	Description of Work
EAR	European Authorized Representative
EC	European Commission
EC PO	EC Project Officer – Scientific Officer
EGF	Endothelial Growth Factor
ELISA	Enzyme-Linked Immunosorbent Assay
EM	Exploitation Manager
FGF	Fibroblast Growth Factor
FGF-b	Basic Fibroblast Growth Factor
GA	General Assembly
GPRS	General Packet Radio Service
ISF	Interstitial Fluid
IST	Information Society Technologies Programme
LEAD	Lower Extremity Arterial Disease
LED	Light Emitting Diode
MDD	Medical Device Directive 93/42/EEC
Mil	Milestone
Min	Minutes
MIPS	Million Instructions per Second
MM	Man Months
mRNA	Messenger Ribonucleic Acid
NA	Non applicable
NMP	Nanotechnology and nanosciences, knowledge-based multifunctional materials and new production processes and devices
PCP	Partner Contact Person

Item	Description
pH	Potential Hydrogen, a logarithmic measure of hydrogen ion concentration or activity: $\text{pH} = -\log[\text{H}^+]$
PM	Project Manager (Coordinator) – CSEM.SA
PCP	Partner Contact Person
PDGF	Growth factors: Platelet-Derived Growth Factor
PHB	Project Handbook
PPR	Project Progress Report
Project Website	http://www.biotex-eu.com
RH	Relative Humidity
TBF	To be found
TBC	To be confirmed
TBD	To be defined
TBF	To be found
TGF- β	Transforming Growth Factor β
TMG	Technical Management Group
VEGF	Vascular Endothelial Growth Factor
WIOS	Wavelength Interrogated Optical System
WP	Work Package
WPL	Work Package Leader

2. Fluids of interest

Since the BIOTEX project aims to measure biological parameters, it is imperative to understand the liquids wherein these parameters are found and the methods to collect them. This section presents the fluids wherein these parameters will be measured.

- Sweat for diabetes and sportsmen applications: fluid on the surface of the body, it contains some substances which are relevant to monitor either diabetes or sportsmen during exercise.
- Exudate for wound healing application: it is the name of the wound liquids which contain several substances measurable in-situ.

Blood is the third body fluid of interest for the foreseen monitoring. This fluid will not be described, since it will be targeted only by oximetry, which is a non-invasive measurement method requiring no contact with the fluid itself. The BIOTEX project aims in this case to integrate this technique into a textile patch.

2.1 Sweat

Several studies on sweat were carried out in the sixties but were discontinued because this body fluid is not very easy to collect. This is mainly due to variable sweat rate and large composition differences between people. Sweat was nevertheless chosen for BIOTEX because it is the main body fluid at the surface of the skin which can therefore facilitate non-invasive measurements. This section gives a description of its main components.

Sweat is secreted by two types of sweat glands, namely eccrine and apocrine glands:

- The eccrine sweat glands are far more numerous. They are distributed over nearly the entire body surface, with a greater density on the soles, the forehead, the armpit, the palms and the cheek.
- The apocrine glands are bigger but less numerous, and are located in hairy regions like the groin, anal regions, armpit, areola in the beard area.

These two types of glands have quite different characteristics and functions. Since the former plays an essential role in the regulation of the body temperature while the latter is suspected to be the source of chemical signals (pheromones) that plays an important role in the reproductive biology of mammals. Far more information is available about eccrine sweat, while apocrine sweat composition is still not well known.

Sweat includes 98-99% of water. Its composition is not well known. Eccrine sweat is a diluted aqueous solution; its weight is represented by 75% of inorganic compounds (mainly sodium, chloride and potassium) and 25% of organic compounds like urea, lactate, ammonia, glucose...).

The composition of sweat can vary extensively between different people. Age, emotional state, diet, exercise and a variety of hereditary factors influence the composition.

The electrolyte concentrations in sweat are affected by the sweat rate: a low sweat rate can cause an increase of electrolyte concentration; an elevated electrolyte concentration can become pathologic.

Sweat composition can change according to the stimulation method, collection, sweat rate and anatomic situation. The sweat rate increases when electrolyte concentration increases. Values for the whole body are:

- At rest: between 0.5 and 0.7l/day
- In a mild heat atmosphere (36°C; 30% relative humidity): 0.8 l/h
- During exercise: 1.5 at 3.4l/h

2.2 Exudate

Exudate is the name of the main fluid of interest for the wound healing application. It is composed of water and plasmatic proteins [6][7]. This liquid is produced by acute and chronic wounds. Usually pale yellow, it can become green in case of pyocyanic proliferation or thick ichor. Neither the composition, nor the regulation and nor the precise significance of exudate in wounds are well known. Indeed, the right balance between keeping a wound wet enough for its cicatrisation and having too abundant exudate is not known.

Exudate comes from blood by extravasation starting from vessels on the level of the wound bed. It is released in wound under the dependence of chemical factors like histamine. The number of studies evaluating exudate production in function of the type of wound is rather limited; exudate reaches 5000g/m²/day in leg ulcer or burns. To have a normal cicatrisation, the quantity of exudate must decrease during cicatrisation but not totally disappear. An increase of its quantity must be considered as a secondary infection.

Exudate composition changes during the wound evolution and contains in variable proportions:

- Blood elements: platelets, red blood cells, leucocytes (which clean the wound and eliminate pathologic germs)
- Electrolytes: sodium, potassium, chloride (concentration similar to plasma)
- Lactates, urea, creatinine, glucose at very low levels
- Albumin
- Proteolytic enzymes: collagenase, elastase, metalloproteinase and lysozyme
- Growth factors: Platelet-Derived Growth Factor (PDGF), basic Fibroblast Growth Factor (FGF-b), Vascular Endothelial Growth Factor (VEGF)

Growth factors are more numerous in acute wounds than in chronic wounds after the detersion phase. Exudate containing growth factors has therefore to be preserved and a wound should not be washed during the detersion phase. However, occlusive wound-dressing are useful to maintain growth factors in contact with the wounds.

- Germs: staphylococcus and pyocyanic (at detersion phase especially)
- Dead cells: products of cell decomposition which can increase exudate viscosity and cause unpleasant odours.

It is very important to evaluate the quantity and the quality of exudate: too large values could be a sign of infection, while too small values could be a sign of necrosis (skin destruction). Work will be carried out during the BIOTEX project to determine the optimum quantity of this fluid to have a normal healing.

3. Sensor types

Several types of sensors will be developed to measure the relevant parameters of the different applications.

These sensors will be:

- Optical sensors for specific chemicals concentration, and pulse oximetry evaluation,
- pH sensors,
- Electrochemical sensor, for electrolyte concentration measurement,
- Breathing and piezoresistive sensors,
- Heart activity sensors,
- Sweat rate sensor,
- Sweat conductivity sensors,

These sensors, depending on the applications will be placed at the most relevant places, taking into account the pathologies, and the textile technical requirements.

Moreover, for sweat which is one of the main fluids to be studied, a specific collecting system will be developed. It will ensure that the sensor will have enough sweat to measure the relevant parameters.

4. Selected applications

4.1 Obese children/sportsmen application

4.1.1 Description of the application

The sensors that will be developed by the BIOTEX project will allow non-invasive sensing of electrolyte concentration, sweat rate and pH of sweat. Beside these chemical sensing, cardiac and respiratory functions will also be monitored.

As physical activity plays a major role in the treatment of obesity, the applications for exercise physiology are also of relevance for studies in obese children. Therefore the sports applications and obesity studies have been grouped together given that they involve assessment of similar physiological parameters. Initial trials for sports applications will be carried out under controlled conditions – in a room at 19-20°C with normal/active healthy subjects. This will allow comparisons with other populations including obese children. All trials will be reproducible, i.e. at a specified percentage of maximum heart rate, therefore the physiological functions in these populations can be assessed over time and may be used to monitor the success of various dietary or exercise regimes.

4.1.1.1 Description of childhood obesity application

In many obese adults, the roots of their disorder often traces back to childhood. Obese children are more likely to become obese adolescents, who are then likely to remain obese as adults. Adult obesity is associated with significant morbidity including hypertension, Type 2 diabetes mellitus and some forms of cancer. Therefore evaluation of obesity in childhood offers the best hope for preventing the progression of this condition and its associated morbidities into adulthood. In addition obesity can have a negative impact on the self-esteem of children and may have significant implications on them psychologically through their lives.

While obesity in childhood leads to increased morbidity in later life, there are many adverse health affects which affect obese children. Obesity is associated with various cardiovascular risk factors and heart failure [Olivares *et al*, Zwiauer *et al*]. Therefore special monitoring of cardiac function is important for obese children. Other conditions include asthma, Type 2 diabetes, hypertension, orthopaedic complications and sleep apnea.

4.1.1.2 Description of sport application

The field of sports science and human performance involves the study of exercise and sports physiology. This area of study examines the scientific basis for understanding how the body responds to single and multiple bouts of exercise. It provides opportunities for the study of biological responses to physical activity across the lifespan. A detailed assessment of physiological functions can allow a physiologist to pinpoint correct training levels and intensities and to assist the coach in prescribing individual training programmes. For sports applications, the development of wearable sensors that are non-obtrusive will allow the assessment of an athlete's performance in the field. Naturally this would provide a better assessment of performance abilities than trials carried out in a laboratory setting.

It is well known that exercise physiology relates to sport performance; however, in the last few decades it has become apparent that the study of exercise physiology is also relevant in clinical settings. Exercise can be used in both the treatment and prevention of multiple diseases including cardiovascular diseases, pulmonary diseases, diabetes mellitus, and several types of cancers.

Relevant parameters

4.1.1.3 Relevant physiological parameters and measurement

The main physiological tests for exercise physiology include percentage body fat (%BF), oxygen consumption (VO_2), maximal oxygen consumption (VO_{2max}), carbon dioxide production (VCO_2), and anaerobic threshold.

- %BF – Percent body fat
- VO₂ – Oxygen consumption
- VO₂max – Maximal oxygen consumption (aerobic capacity)
- VCO₂ – Carbon dioxide production
- HR – Heart rate
- LA – Blood lactate (lactic acid) and anaerobic capacity
- pH of sweat

4.1.1.4 Relevant parameters in sweat

As for the previous application, one of the key fluid to sue is sweat. The most interested parameters are :

- Sodium, potassium, chloride [24].
- Zinc [25]
- pH [26-29]

4.1.1.5 Pulse oximetry (haemoglobin oxygen saturation)

Obese children

The risk of acute hypoxemia during induction of anaesthesia in obese patients is a well known hazard, with a mean value of 80% at one minute of exercise when the patient is obese. The countermeasure is to preoxygenate all patients before induction of anaesthesia. Monitoring of haemoglobin oxygen saturation (SpO₂) for obese children is considered on this basis to be a routine precautionary measure.

Sportsmen

It was recently shown [30] that all athletes practising endurance trials experience hypoxemia before the maximal level of exercise. This transient hypoxemia is characterized by a fall of oxygen haemoglobin saturation (SpO₂), due to an alveolar hypoventilation. When the exercise continues and for maxima levels located between 80 and 100% VO₂ max, some athletes find their initial saturation level while others worsens their desaturation and then develop a hypoglycaemia induced by the exercise. To control the oxygen saturation, an oximeter with an ear sensor is used.

The sports which mobilized a high muscular mass induce a higher extraction of oxygen in muscles. For example: 100% of oarsmen develop hypoxemia induced by exercise whereas this figure is halved to 50% for cyclists and runners. A study has shown that all the tested oarsmen presented an exercise induced hypoxemia with a SpO₂ ranging between 5 and 11%.

4.1.1.6 Respiration rate

The respiration rate [31][32][33] is the number of breaths a person takes per minute. The rate is usually measured at rest and simply involves counting the number of breaths for one minute, i.e. how many times the chest rises per minute. Respiration rates may increase with fever, illness, and with other medical conditions. Normal respiration rates for an adult person at rest range from 15 to 20 breaths per minute. Respiration rates over 25 breaths per minute or under 12 breaths per minute (at rest) may be considered abnormal. During exercise, respiration rate can increase up to 25-30% above resting rate.

4.2 Diabetes

4.2.1 Description of the application

Diabetes [34][35] is probably the most significant disease in which the daunting task of management is really placed in the hands of patients and their relatives. Diabetics have to balance the ups and downs of blood glucose levels throughout the day and night. Those

levels can be affected by meals and exercise. Since the 1980s, the only available home test was the frequent use of finger sticks. Despite the enormous effort spent in research for glucose sensors (implantable and/or portable), only a few years ago some glucose monitoring systems appeared in the market. It would not be realistic for the BIOTEX project to run through again the same hard way, and a holistic multi-parametric approach seems more promising. Diabetes is a difficult disease to control, therefore any system allowing the monitoring of relevant parameters and supporting prompt decisions would raise an enormous interest.

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β -cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, and to what extent, is the primary cause of the hyperglycaemia.

The disease is classified into several categories:

- Type 1 diabetes, formerly known as insulin-dependent diabetes mellitus or juvenile-onset diabetes mellitus, is caused by autoimmune destruction of the β -cells of the pancreas, rendering the pancreas unable to synthesize and secrete insulin.
- Type 2 diabetes mellitus, formerly known as non-insulin dependent diabetes mellitus or adult-onset diabetes mellitus, results from a combination of insulin resistance and inadequate insulin secretion.
- Other types of diabetes which are rarer.

Type 2 is the most common form, accounting for 90-95% of diabetes in the developed countries.

In 1998 the study Code-2 (Costs of Diabetes in Europe – Type 2) was carried out in 8 EU countries (Belgium, France, Germany, United Kingdom, Italy, Holland, Spain, Sweden) to assess costs of management of diabetic patients. This study pointed out that diabetes is a pathology with high social and economic costs. The value of economic resources devoted to diabetes care accounted for 5-10% of the overall health budget in these EU countries (6.65% in Italy, about 5000 billion Euros). The cost per person resulted in about 3000 €/year, almost the double of average people. Interestingly, 39% of these costs were due to treatment of complications and 29% to direct treatment of the diabetes. Oral medications for diabetes and insulin accounted for only 10% of the overall pharmaceutical expense of these patients.

As the pathology is diagnosed, oral medications are firstly used to control blood glucose level and stimulate insulin production:

- *Sulfonylureas*, to stimulate insulin production and secretion by the pancreas;
- *Biguanides*, to decrease glucose production by the liver and lessen the insulin resistance by the body;
- *Glucosidase inhibitors*, to prevent the absorption of sugar by the bowel;
- *Thiazolidinediones*, primarily to make the body respond to insulin (lessens insulin resistance) and to decrease the production of sugar by the liver;
- *Meglitinides*, to stimulate the release of insulin by the pancreas.

When this approach becomes insufficient to control the diabetes, insulin is used (many types are available with different action times).

Many questions concerning diabetes are still waiting for an answer (pathogenesis, alterations in metabolism, therapy) and many problems lack solutions. The onset of Type 2 diabetes is

estimated to occur about 4 to 7 years before clinical diagnosis, and epidemiological evidence indicates that complications may begin several years before clinical diagnosis. For this reason, fast non-invasive screening methods are needed. The management of the pathology is a complex task and it is the patient to shoulder large part of the burden: there is a need for new effective and reliable monitoring devices. For its characteristics, BIOTEX project is most suited to develop a monitoring device more than a diagnostic method.

4.3 Wound healing/skin graft

4.3.1 Description of the application

The wound healing involves many parameters; the process is neither well controlled by doctors and nor well understood by researchers. Furthermore, there are great variations in the healing time of wounds.

The BIOTEX project aims at monitoring the wound healing process and measuring the physiological parameters playing an essential role, as for example growth factors. The idea is to keep both patients and doctors informed about wound evolution and complications.

The sensor will consist in a patch incorporated in a textile (garment or wound dressing), and will be placed in contact with the wound from the first day on. It will allow monitoring the injury evolution either in hospital for any wound size or at home for small ones.

Several ways to use this sensor can be considered depending on the wound gravity. For serious wounds, doctors will be able to use these sensors to monitor the evolution in real time and quickly adapt the treatment when necessary. For patients staying at home, they will be able to control the way the healing process is taking place and inform the doctors or nurses in case of trouble. This innovation would reduce the number of serious pathologic wounds (chronic wounds like ulcer necroses) which are more difficult to heal.

4.3.1.1 Mechanism of wound healing

Human and animal tissues are able to cure wounds by processes of repair and regeneration. The speed and the quality of the wound healing depend on:

- The general state of the affected body
- The state and the localization of the wound
- The presence or absence of an infection.

The treatment and the care of a wound can therefore not be simply schematized. Even in the presence of hurts of identical aetiology, the evolution of the healing process can be totally different depending on the person.

The cicatrisation after a tissular lesion appears when the platelets come into contact with exposed collagen. The healing mechanism can be detailed as four stages:

1. **Deterision:** 4 to 5 days, the growth factors secreted by the platelets arrive on the first day. They begin to produce fibroblasts cells.
2. **Granulation:** during three weeks, the fibroblasts synthesize collagens under the influence of growth factors. During this stage, the fibroblasts change into myofibroblasts which are muscular cells. The myofibroblasts secrete a chemical substance which prevents their death. Collagen is therefore not destroyed and causes a tumefaction.
3. **Epidermisation:** the skin is formed. There is less growth factor secreted.
4. **Cicatrisation:** this stage lasts about one year to obtain a solid skin.

If problems occur during the cicatrisation process, the healing is delayed or the wound may become chronic. Many psychopathological factors may delay cicatrisation, such as:

- Infection: it consists in an invasion of cutaneous and under cutaneous tissues by germs (multiresistant bacteria to antibiotics). There may be some clinical signs such as local ignition and/or abnormal flow of liquid
- Diabetes, malnutrition and age are responsible of the deceleration of the cicatrisation of the wound.

4.3.1.2 The different types of wound

Two families of wounds are distinguished: acute wounds and chronic wounds. They are described in the following subsections.

Acute wounds

They are created by a traumatic agent on a healthy tissue. The healing takes place normally if the tissues are correctly vascularised.

Chronic wounds

Wounds that are older than 4 months are called chronic wounds:

- *Venous ulcer*: leg ulcer is characterized by a chronic substance loss on the leg. For 90% of the ulcers, it is caused by a chronic venous deficiency associated in 25% of the cases to an arterial reaching. Its spontaneous evolution is traduced by absence of cicatrisation, extension of healing duration and secondary infection.
- *Eschar (decubitus ulcer)*: the mechanism of eschar formation is caused by tissular hypoxo. It is due to a pressure applied between bones and a hard surface (e.g. bed or armchair). The pressure causes a vascular occlusion, then an ischemia, and finally an irreversible tissular necrosis.
- *Diabetic wounds*: these wounds are due to a lack of vascularisation at the level of foot and become chronic wounds.
- *Chirurgical wounds*: aftereffects resulting from surgery can take a long time to heal. These wounds have to be carefully monitored.
- *Burns*: there are 3 degrees of burns:
 1. *First degree*: only the epidermis is touched, it is an acute wound
 2. *Second degree*: injury can be superficial or deep
 3. *Third degree*: a skin graft is necessary because the skin basal membrane is destroyed.

4.3.2 Relevant parameters

The cicatrisation is characterized by many markers; the relevant ones are described in the following. Three types of cells are of interest for this description:

- Keratinocytes: cells able to proliferate for the production of a dead cell
- Fibroblasts: role of mechanical supports, secrete the extra cellular matrix elements.
- Endotheliocytes: constitute the wall of the vessels; produce the components of the basal membrane

4.3.2.1 Components of the extra cellular matrix

The extra cellular matrix is made of collagenous fibres comprising collagen macro molecules, elastin and fibroectine, associated with a fundamental substance made of glycosaminoglycans (in particular the hyaluronic acid). These components of extracellular matrix are secreted by fibroblasts and keratinocytes under the influence of the growth factors (especially the Transforming Growth Factor).

It is expected that the measurement of these parameters will not be a simple task and no information was found on how to do it.

4.3.2.2 Growth factors

Growth factors are small, often glycosylated proteins that are responsible for cellular proliferation. They self-attach to specific receptors on the surface of a target cell and promote differentiation and maturation of these cells. They act by autocrine, paracrine or endocrine mechanisms and are generally present at small concentration in wound environment. Currently, the typical concentration of the growth factors is not well known because it changes during the different stages of wound healing, but ranges from pg/ml to ng/ml of tissue.

The most important growth factors are the EGF (Endothelial Growth Factor), PDGF (Platelet-Derived Growth Factor), TGF- β (Transforming Growth Factor β), VEGF (Vascular Endothelial Growth Factor) and the FGF (Fibroblast Growth Factor), which stimulate the proliferation of endothelial cells, fibroblasts, basal keratinocytes and fibroblasts. These growth factors are mainly secreted by platelets.

The normal concentration of the growth factors is not well known because it changes during all different stages of healing: it ranges between ng/ml and pg/ml of tissue. A growth factor of particular interest is the vascular endothelial growth factor (VEGF), essential for optimal wound angiogenesis as it promotes the development of new blood vessels. While the ultimate goal of VEGF is to increase the amount of nutrients provided to the tissue and the rate of removal of waste products, it has been shown to stimulate vasodilatation, angiogenesis, and increase microvascular permeability (however, almost identical isoforms of VEGF are doing the opposite). Detection of VEGF aims at gaining information on the evolution of the wound healing process. In addition, diminished production of VEGF in diabetic patients lead to decreased angiogenesis and partly explains impaired tissue repair in diabetic patients. Generally detection of VEGF is made through the expression of VEGF mRNA. However, this is not always accurate and a direct detection of the growth factor itself would be more reliable.

Measurement techniques of growth factors

All growth factors currently known were detected initially by their biological activity, which allows the measurement of their concentration in several environments. Since growth factors are proteins, antibodies can be produced and immunologic techniques adapted to their quantification can be developed. The two most used methods are Immunoassay and Enzyme Linked Immunosorbent Assay (ELISA) which use a first antibody for the capture of antigen growth factor and a marked second antibody for its revelation.

ELISA [50][51] is used to detect the presence of an antigen, such as a disease-related agent, from a sample of body fluid. This method combines the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. ELISA can provide a useful measurement of antigen or antibody concentration. There are many different types of ELISA. One of the most common types is "sandwich ELISA" which measures the amount of antigen between two layers of antibodies. The antigens to be measured must contain at least two antigenic sites capable of binding to antibodies, since at least two antibodies act in the sandwich. Sandwich assays are thus restricted to the quantisation of multivalent antigens such as proteins or polysaccharides. Sandwich ELISA is valuable when the concentration of antigens is low and/or when they are contained in high concentrations of contaminating proteins.

ELISA is a useful and powerful method in estimating about 1 ng/ml to 1 pg/ml materials in the solution, such as serum, urine and culture supernatant. It is a simple task to make ELISA if there are "good" antibodies against the concerned materials such as proteins, peptides and drugs. These tests come in kit forms with coating microtiter wells. The method is however indirect and the results are therefore not available immediately.

4.3.2.3 Bacteria/pH

The bacterial colonization is a natural phenomenon [52] necessary for cicatrisation. There is a risk of infection when the number of bacteria increases excessively. It is therefore important to monitor the level of bacteria to diagnose an infection and to prevent delay in cicatrisation delay.

The bacteria cycle is the usual support of bacterial development on a wound (Figure 1). The different cicatrization stages correspond to the succession of physiological bacterial flora of colonisation. In this bacteria cycle, the complication phases 3 and 4 are:

- Ulceration (phase 3), wounds are low in germs
- Necrose (phase 4), Gram- germs appears

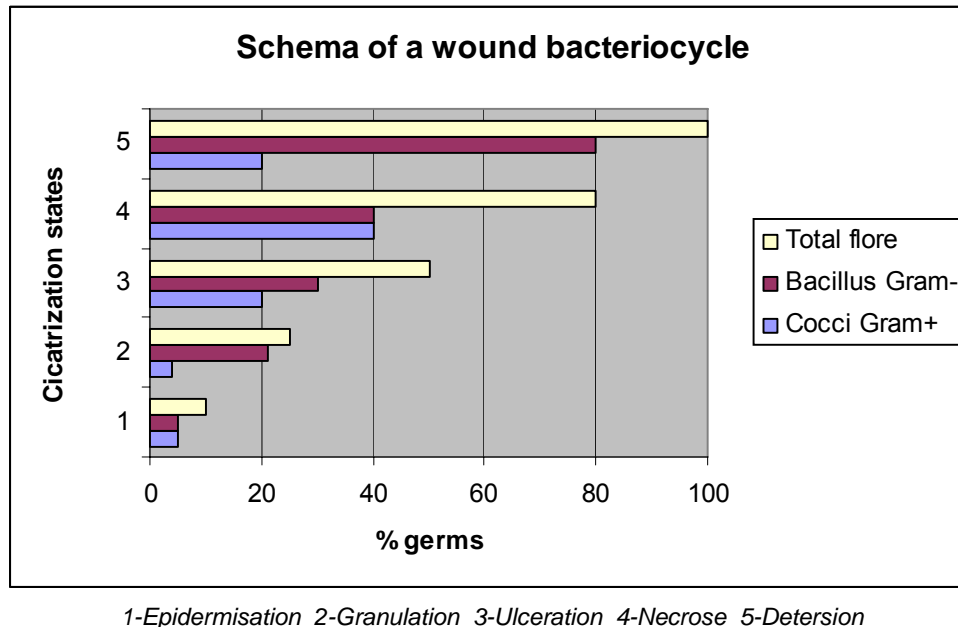


Figure 1: Evolution of bacteria during the wound healing process: phases 3 and 4 are pathologic states

Currently, the only recognised direct ways to determine the amount of bacteria are either by biopsy or by sampling with a sterilized cotton shank. The threshold of 10^5 CFU per gram of tissue indicates an evolution towards infection.

Since the amount of bacteria changes the pH in a correlated way, it represents another way to measure the presence of bacteria. Infection by Gram+ or Gram- bacteria can also be found by pH measurement.

4.3.2.4 Histamine

Release of histamine by leukocytes is a signal for allergic reactions. Monitoring amount of histamine will allow checking if the cicatrization speed is fast enough or too slow. Histamine steps at the beginning of wound healing process during the inflammatory phase. After a few days its concentration decreases. The patient would be warned if the histamine concentration increases in an undesirable way. Concentration of histamine in most body fluids is as low as 3-10 nM, 200-2000 nM and 30-100 nM in plasma, blood and urine, respectively. In wounds, histamine is a proinflammatory factor released by blood platelets during the inflammatory phase (first phase) of wound healing [52]. The concentration of histamine in wounds (between 10 and 100nM) has been shown to be proportionally indicative of inflammatory reactions, and therefore would be of interest to detect in situ, what has, to our knowledge, not been successfully achieved until now. Detection of histamine would be possible providing we have a specific antibody (commercially available), using reversible swelling of hydrogels networks grafted with either histamine or antibody. We have so far worked on the ex situ detection of a derivative of histamine, and could detect concentrations as low as 20 nM. Detection of histamine in wounds with hydrogel-based sensor is innovative and seems promising. But it is not clear yet how large the variations must be to be clinically significant (to be defined during the project).

4.3.2.5 Others species in presence in exudate

Other present species are:

- Blood elements: platelets, red blood cells, leucocytes (clean the wound and eliminates pathologic germs)
- Electrolytes: sodium, potassium, chloride (concentration similar to those of plasma)
- Lactates, urea, creatinine, glucose at very low levels
- Albumin
- Proteolytic enzymes: collagenase, elastase, metalloproteinase, lysozyme

Other growth factors like Platelet-derived growth factor (PDGF), basic Fibroblast growth factor (FGF-b) are in presence in wounds. Currently, more than fifteen growth factors have already been discovered and researchers think that others have still to be found.

Complexity of the biological medium present in wounds (exudate) requires a preliminary analysis of parasite species that might cross-react with the sensor to insure sensor selectivity. This can be tailored with the selectivity of the chosen antibody. Cross-reactivity might be expected for similar growth factors.

4.3.2.6 Hydrogen rate

Sensors sensitive to the passage of hydrogen allow detecting the presence of septicaemia or bacterialisation. They are available on the market.

4.4 Implant rejection

4.4.1 Description of implant rejection application

Despite outcome of liver transplant (LT) recipients has steadily improved over the recent years, thanks to progress in surgical techniques and better profile of immunosuppressive agents. Graft dysfunction (GD) still remains a major issue facing both patients and transplant physicians alike. By GD, we define a set of liver graft diseases that range from acute rejection (AR), to chronic allograft diseases (CAD), to initial poor function (IPF), to delayed graft function (DGF), to primary non function (PNF), to native liver disease recurrence or relapse (such as in the event of HCV+ recipients). In the early post-transplant course, incidence of AR may range as high as 67% in the international literature, according to time to diagnosis and immunosuppressive regimen, while DGF may affect up to a quarter of LT recipients, according to type of graft and donor selection criteria. On the opposite, major issues in the late post-transplant course are CAD and native disease relapse, especially in the setting of HCV-related cirrhosis. Recent data on extended criteria donors correlate donor age with HCV relapse, and due to the current shortage of donors, post-transplant HCV relapse is bound to affect an ever increasing percentage of LT recipients. Thus, the need for constant monitoring of LT patients is ever more relevant, since timely diagnosis and treatment of post-transplant morbidities may positively affect outcome and reduce complication rates. Therefore, it is highly advisable that a system for constant monitoring of vital parameters and liver function be designed and put in practice to allow prompt detection of post-transplant complications. Once alterations in vital parameters and/or liver function tests are detected, patients may be referred for immediate consultation with a transplant physician and second-line investigations. A method of non-invasive monitoring of liver function and vital parameters might replace scheduled, random follow-up investigations and help focus physicians on patients with truly acute and/or chronic liver function impairment.

4.4.2 State-of-the-art of the application

Monitoring of vital parameters and liver function in LT patients is currently carried out by frequent blood analyses of transaminases, bilirubin and few other parameters. Once deviations are identified, second-line investigations are activated consisting in the assessment of immunosuppressor blood levels (calcineurin inhibitors, proliferation signal

inhibitors, antimetabolites, etc...), liver ultrasound and/or biopsy, which are necessary for a correct diagnosis. This type of monitoring activity afflicts patients for a long time causing a substantial depletion of quality of life. At the moment, all these tests are carried out at lab level; no sensor technology or chemical test strips exist allowing home testing.

Publications reporting studies concerning possible modifications of sweat composition as a consequence of poor liver function are not available. A device performing therefore non-invasive monitoring of sweat composition could be useful for both research and clinical purposes.

5. Quality Assurance, standards and regulations

Although BIOTEX's project aims at the feasibility of integrating biosensors into textiles by making prototypes to perform the necessary verifications, some regulation aspects have to be respected from the beginning of the project to ensure subject safety during trials (some sensors will also be tested in-vivo) and to not delay the realization of future products after the project.

BIOTEX results will clearly belong to medical devices, which are defined as “*any article or healthcare product intended for use in diagnosis or disease or other condition or use in the care, treatment or prevention of disease*” [Directive 93/42/EEC]. Furthermore, BIOTEX does not belong to drugs since it does not achieve any of its primary intended purposes by chemical action or by being metabolized. Regulation of medical devices is intended to protect the consumer's health and safety by attempting to ensure that marketed products are effective and safe. Two tracks could be followed, mainly based on the markets that future products aim at:

- The European CE marking for medical devices, which governs Europe and other countries.
- The Food and Drug Administration (FDA) rules (also) the approval of medical devices for United States of America; the approval is also recognised by other countries.

The case of products to be put on the market and the case of devices for investigation purpose / custom-made have to be treated separately. The current BIOTEX project is concerned by the latter case, while a future product resulting from the work started in this project should comply with the former one.

5.1 Relevant standards

Beside the Medical Device Directive 93/42/EEC from which the information provided above are derived, other standards have to be fulfilled. In summary:

- The conception must fulfil quality assurance:
 - ISO 9000:2000 Quality management system
 - ISO 13485 Quality management and corresponding general aspects to medical devices (replacing progressively EN 46000 series)
- The conception must respect the user security:
 - EN1441 Medical devices – Risk analysis and EN ISO 14971 Medical Devices – Application of risk management to medical devices. Define the probability and risk of each hazard using either a bottom-up Failure Mode and Effect Analysis (IEC 60812) or a top-down Fault Tree Analysis (IEC 1025)
 - EN60601-1-4 Medical electrical equipment Part1: General requirements for safety 4: Collateral Standard: Programmable electrical medical systems
- These products are in contact with skin (hurt or healthy). The biocompatibility will be evaluated according to norm EN ISO 10993-1.
- If textile is realised with standard fibres (used for garments), requirements conformity like “oekotex” or “Ecolabel” will be considered.
- Data transmission should comply with ETSI standards. Since already certified Bluetooth communication modules will be used, only partial testing of the communication is required; e.g. tests will concern user security, device operation immunity to external electromagnetic fields, radiated power.
- Electromagnetic testing, e.g. EN 60601-1, is applicable and compliance will have to be verified.

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