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BIO-ARTIFICIAL LIVER SUPPORT SYSTEM:
An evaluation of models used in demonstrating or improving metabolic and clinical efficacy

by
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ABSTRACT

Acute liver failure (ALF) is a rare but devastating clinical syndrome with multiple causes and a variable course. The mortality rate is high. Orthotopic liver transplantation is the only therapy of proven survival benefit but the limited supply of donor organs, the rapidity of progression and the variable course of ALF limit its use. A need therefore exists for a method to 'bridge' patients, that is, provide temporary support, to either the spontaneous regeneration of the innate liver or transplantation. One possibility includes bio-artificial liver support systems (BALSS). This technology is composed of an extracorporeal circulation system incorporating a bioreactor that contains parenchymal liver cells (hepatocytes) to perform the detoxifying, transforming and synthetic properties of a liver. However, the development of a BALSS holds particular challenges. Despite approximately four decades of research, bio-artificial liver (BAL) technology globally remains in a pre-commercial stage. The University of Pretoria (UP) and the Council for Scientific and Industrial Research (CSIR) have developed a BALSS with novel characteristics. These include a computationally optimized radial-flow primary porcine hepatocyte bioreactor perfused with blood plasma, and a perfluorocarbon oxygen carrier which replaces hemoglobin. There are also novel design properties in the circulation system itself. Demonstrating the metabolic and clinical efficacy of a BAL device requires implementing, *in vitro* (cell biology), *in vivo* (animal) and mathematical modeling studies. These studies are a formal necessity but are inherently 'models' of the *in vivo* human clinical circumstance. That is, they are limited by their experimentally controlled configuration/s. In investigating these, this thesis firstly provides a foundation by reviewing the clinical and biological context of ALF and BAL technology, then presents and evaluates particular studies/models that have been implemented over several years in the course of the UP-CSIR BAL project. For each section, thoughts and recommendations regarding future work that will facilitate the development of BAL technology are discussed in detail. The thesis is concluded with an evaluation of success and the consensus-agreed requirement of continued research and innovation in the field.

Keywords: acute liver failure, bio-artificial liver, hepatocyte bioreactor cell biology, animal models, compartmental pharmacokinetic models, prognosis modeling, bioprocess monitoring, state estimator.

BIO-KUNSMATIGE LEWER ONDERSTEUNINGSTELSEL: ’n Evaluasie van modelle wat gebruik is in die demonstrasie of verbetering van metaboliese en kliniese doeltreffendheid

SAMEVATTING

Akute lewerversaking (ALV) is ’n seldsame maar vernietigende kliniese sindroom met veelvuldige oorsake en uiteenlopende nagevolge. Die sterftesyfers is hoog. Leweroorplanting is die enigste terapie met bewese oorlewingsvoordele, maar die tekort aan oorplantingsorgane en die verskeie nagevolge van ALV beperk die gebruik daarvan. Daar is dus ’n behoefte aan ’n ‘oorbruggingsmetode’, om pasiënte te ondersteun terwyl spontane regenerasie van die bestaande lewer kan plaasvind, of voordat ’n leweroorplanting gedoen word. Een so ’n moontlikheid is ’n bio-kunsmatige lewerondersteuningstelsel (BKLOS). Hierdie tegnologie is ’n buiteliggaamlike sirkulasiestelsel insluitende ’n bioreaktor wat lewerselle (hepatosiete) bevat wat die suiwering, transformasie en sintese eienskappe van die lewer vervul. Die ontwikkeling van ’n BKL-ontwerp hou definitiewe uitdagings in. Ten spyte van vier dekades se internasionale navorsing, bly bio-kunsmatige lewer tegnologie in ’n pre-kommersiële stadium. Die Universiteit van Pretoria (UP) en die Wetenskaplike en Nywerheidnavorsingsraad (WNNR) het ’n BKLOS met uitsonderlike kenmerke ontwikkel. Dit sluit in ’n rekenaar-geoptimiseerde radiaal-vloei primêre vark-hepatosiet bioreaktor wat deurentyd gevul word met bloedplasma en ’n perfluorostikstof suurstofdraer wat hemoglobien vervang. Die sirkulasiestelsel self het ook unieke ontwerpeienskappe. Die demonstrasie van die metaboliese en kliniese doelteffendheid van ’n BKL-ontwerp vereis die implementering van *in vitro* (selbiologie), *in vivo* (diere) en wiskundige modeleringstudies. Alhoewel sulke studies noodsaaklik is, is hulle inherent ‘modelle’ van die *in vivo* menslike kliniese omstandigheid. In hierdie proefskrif is eerstens ’n basis gebou deur die kliniese en biologiese samehang van ALV en BKL tegnologie te ondersoek. Daarna stel dit voor en evalueer spesifieke studies/modelle wat oor verskeie jare in die loop van die UP-WNNR BKL projek geïmplementeer is. Na elke afdeling is voorstelle bespreek in verband met toekomstige werk wat die ontwikkeling van BKL tegnologie sal vergemaklik. Die proefskrif word afgesluit met ’n evaluering van suksesse tot op datum asook behoeftes aan voortgesette navorsing en ontwikkeling in die veld.

CONTENTS PAGE

LIST OF FIGURES

LIST OF TABLES

LIST OF TERMS AND ABBREVIATIONS

The majority of definitions were taken from the *on-line* medical dictionary of the University of Newcastle Upon Tyne (available at http://cancerweb.ncl.ac.uk/omd/).

Term	Meaning
a priori	previously defined or assumed (facts)
acidosis/ alkalosis	a metabolic condition, characterized by an increase or decrease in hydrogen ion concentration
allocompatibility	immunological compatibility of substance/fluid from another person or organism
average relative accuracy/error region	the product of the measurement range and the standard deviation of the relative error (see below)
bioanalytical/bioprocess monitoring system	a (typically) predictive, *on-line* monitoring system for a contained biological process (normally for commercial purposes)
biochemical	a molecule characterized or produced by chemical reactions in a living organism
bioreactor	a closed device housing cells used for generating/metabolizing biological substances
bioresorbable	a material that may be metabolized/absorbed when inserted into an organism's tissues
biosensor	a sensor for particular biological stimuli in a bioprocess
bio-systems model	a computational representation of a biological system or bioprocess
boolean switching net	a network of switches employing Boolean rules
bootstrapping	a statistical method for generating theoretical data where limited or no measured data is available
cardiac arythmia	an irregular electrical pattern of cardiac activity
cell aggregation/support matrix	a synthetic or biological three dimensional matrix into which aggregation dependent cells are seeded
chemokine	cytokines that are chemotactic for leucocytes (e.g. IL-6)
disseminated intravascular coagulopathy	a complication of septic shock where endotoxin induces systemic blood clotting, depletion of coagulation factors and thrombocytopenia leading to widespread spontaneous bleeding.
chemotactic	the responsiveness of motile cells to concentration gradients of particular dissolved substances
cytokines	small molecules released by cells and having specific effects on cell-cell interaction, communication and behaviour (e.g. IL-1β)
data driven modeling	modeling procedures based on empirically observed (rather than theoretical) trends in measured data
devascularization	surgical occlusion or all or most of the blood vessels to an organ or tissue
endotoxin	toxic membrane lipopolysaccharides of gram negative bacteria
epileptiform activity	brain electrical activity normally associated with epileptic seizures
etiology	a branch of medical science concerned with the

	causes or origins of diseases
flow injection analysis	the analysis of a chemical substance by removing a sample from a flow stream and mixing it with a reagent to produce a measurable reaction for a detector and subsequent data logging device
gluconeogenesis	the synthesis (mainly be the liver) of glucose from non-carbohydrate precursors
genotype	the total genetic constitution of a cell or organism
glomerular filtration	filtration function performed by the glomerular cells of the kidney
glycolysis	the anaerobic conversion of glucose to pyruvate
haemodynamic	relating to physical aspects of the blood circulation
hepatectomy	the surgical removal of the liver
hepatocytes	the epithelial cells composing (approximately) 80% of the liver
hepatotoxic	toxic to the liver
hepatotrophic	causing liver growth or regeneration
hyper/hypocapnia	an excess or deficiency in carbon dioxide in the blood resulting from hypo or hyper ventilation and leading to acidosis or alkalosis respectively
hyper/hypoglycemia	an excess or deficiency of glucose in the blood
hyper/hypokalemia	an excess or deficiency of potassium in the blood
hyper/hypometabolic	an excess or deficiency in metabolic activity
hyper/hyponatremia	an excess or deficiency of sodium in the blood
hyper/hypophosphatemia	an excess or deficiency of phosphate in the blood
hyper/hypotension	persistently high or low arterial blood pressure
hyper/hypovolemia	increased or decreased blood volume
hyperammonemia	a pathologically increased blood ammonia concentration
hypoxic	a lack of oxygen (low pO_2)
immunosuppression	the suppression of T or B lymphocytes with particular drugs
in situ	in a natural or normal bodily compartment without entering any other place
in silico	a computer-based simulation or model of a system
in vitro	observable in an artificial environment (e.g. a laboratory)
in vivo	within a living body
ischemic	a lacking in oxygen supply to the organs commonly due to a decrease in blood perfusion
Kalman filter	a linear bioprocess observer used to reconstruct the state of a system (including the estimation of non-measurable variables) and to decrease measurement noise. It has time varying observer gain and the descriptive equations are a linear combination of ordinary differential equations.
knowledge-based	a system incorporating clinical knowledge
laparotomy	general abdominal surgery
leucocyte	a member of the group of white blood cells
linearization	a mathematical procedure by which a (non-linear) function is estimated in terms of the expansion of its derivatives at a point of interest
lipohylic/phobic	lipid/fat soluble or insoluble

mass transfer	the concentration gradient of a particular substance at a defined interface
metabolic zonation	the ability of tissues within an organ to adapt to different metabolic requirements
microdialysis	very low volume flow injection method for analyzing biological fluids
mitogenic	the ability of a substance to induce mitosis (division) in eukaryotic cells i.e. growth or regeneration
model	an experimentally controlled simulation of an objective system, circumstance or reality.
model standardization	the reduction of a complex system to particular criteria or rules determining its validity or success
monte carlo analysis	a statistical method in which (bound) random data is generated to overcome limitations in the amount of measured data available (see 'bootstrapping')
necrosis	a form of cell death
nephrotoxic	toxic to kidneys
object oriented model	a model in which the functional units of a system are defined as objects having characteristics that are definable using particular computational methods (e.g. the UML)
Occam's razor	The principle, by which particular predictions of a model are excluded, based on their not being observed in reality. Alternately, 'the simplest explanation is the best'
off-line	not measurable at the time at which a process takes place i.e. results are only available subsequently
on-line	measurable at the time at which a process takes place so that results may potentially be input into a control system
parenchymal/non parenchymal	tissues/cells composing (or not composing) the general functional framework or stroma of an organ
perfusate	a body fluid perfusing a particular stream in a system (e.g. a plasma perfused bioreactor)
periportal	surrounding or close to the intrahepatic portal vein branches (i.e. high oxygen content blood at the inlet of the liver)
perivenous	surrounding or close to the central vein (i.e. low oxygen content blood at the outlet of the liver)
petri net	describes a process in terms of places (circles), transitions (rectangles) and arcs (lines). The formal mathematical semantics used to define discrete distributed systems
pharmacokinetic compartmental model	a closed system of physiological compartments between which the mass transfer (production or clearance) of particular substances may be described using ordinary differential equations (i.e. as defined by Michaels-Menten kinetics)
phenotype	the expressed functional characteristics of a cell or organism resulting from the interaction of its genotype to particular environmental conditions
point error	the difference between corresponding predicted to measured values as a fraction of each measured value

prognostic criteria	particular rules or metabolic indices that may be used to forecast the probable outcome of a clinical intervention or disease process
prophylaxis	preventive treatment for a disease
real-time	sinonymous with *on-line* i.e. data measurable during an experiment or procedure
relative error	a percentile value for the point error divided by the standard deviation of the measured population
software sensor	an indirect computational method for estimating a non-measurable process variable
spectrophotometric detection	a method for determining biochemical concentrations in a sample based on the absorbance/transmission of light at particular wavelengths
splanchnic circulation	blood circulation to the internal organs and lower limbs
state estimator	a system which produces estimates of measurable and non-measurable state variables in a bioprocess
state machine/diagram	a UML graphical representation of the behaviour of a system i.e. the total set of states and transitions through which the system may proceed
stellate cell	Star-shaped, non parenchymal cells in the liver facilitating the phenotypic stabilization of hepatocytes during liver regeneration
systemic	pertaining to the integrated functions of the body as a whole
tornado diagram	a diagram displaying the sensitivity of the outputs of a model in term of its inputs
tumorigenic	tendency to cause cancer
vasoconstriction/dilation	the diminution/enlargement of vascular diameters (especially arterioles) leading to a decrease or increase in blood perfusion to tissues/organs downstream
xenogenic	originating from outside and introduced into an organism (i.e. a foreign material)
zoonosis	the transmission of a disease from an animal or non-human species to a human

Abbreviation	**Meaning**
ABG	arterial blood gas
ABP	arterial blood pressure
AHF	acute hepatic failure (synonymous with acute liver failure)
ALF	acute liver failure
AMC-BAL	Amsterdam Medical College bio-artificial liver
ANN	artificial neural network
ANOVA	analysis of variance
ARDS	acute respiratory distress syndrome
BAL	bio-artificial liver
BALSS	bio-artificial liver support system
BLSS	bio-artificial liver support system
CE	cerebral edema

CFD	computational flow dynamics
CPP	cerebral perfusion pressure
CSIR	Council for Scientific and Industrial Research
CVP	central venous pressure
CVVHDF	continuous veno-venous hemodiafiltration
DIC	disseminated intravascular coagulopathy
ECG	electrocardiogram
EEG	electroencephalogram
EGF	epidermal growth factor
ELAD	extracorporeal liver assist device
FDA	US federal drug administration
FHF	fulminant hepatic failure
GCP	good clinical practice
GFR	glomerular filtration rate
GMP	good manufacturing practice
GUI	graphical user interface
HAL	hepatic artery ligation
HBAL	hybrid bio-artificial liver
HBV	hepatitis B virus
HE	hepatic encephalopathy
HGF	hepatocyte growth factor
HIV	human immuno virus
HRS	hepato-renal syndrome
ICP	intracranial pressure
IL-1β	interleukin-1beta
IL-6	interleukin six
INR	international normalized ratio
IVC	inferior vena cava
K_m	Michaelis constant
MAP	mean arterial pressure
MARS	molecular adsorbents recirculation system
MCA	monte carlo analysis
MELS	modular extracorporeal liver support
MEM	minimum essential medium
MOF	multi-organ failure
NAC	N-acetylcysteine
NO	nitric oxide
OLT	orthotopic liver transplantation
OUR	oxygen uptake rate
PCA	porto-caval anastomosis
pCO_2	partial pressure of carbon dioxide
PET	positron emission tomography
PERV	porcine endogenous retrovirus
PFC	perfluorocarbon
PFOB	perfluorooctyl bromide
pO_2	partial pressure of oxygen
PROM	Prometheus artificial liver support system
PT	prothrombin time
PUF	polyurethane foam
RFB	radial flow bioreactor
SFHF	subfulminant hepatic failure
SIRS	systemic inflammatory response syndrome

SOM	self organizing map
TECA-HAL	TECA-hybrid artificial liver support system
TGF-α	transforming growth factor alpha
TNF-α	tumour necrosis factor alpha
UML	unified modeling language
UP	University of Pretoria
UPBRC	University of Pretoria Biomedical Research Centre
V_{max}	Michaelis-Menten kinetics V max value