UNIVERSITÉ DE TECHNOLOGIE COMPIÈGNE

BIOLOGICAL ENGINEERING

UMR CNRS 6600 Biomechanics and BioEngineering

Hepatocytes microencapsulation for medical application : Bioartificial Liver

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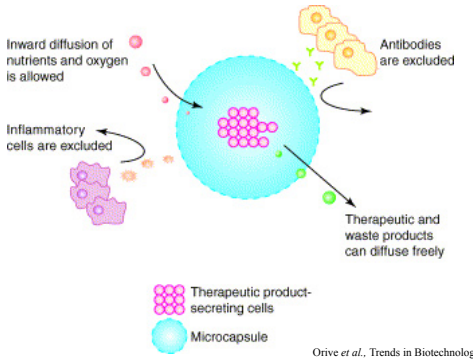
Microencapulation parameters

Material
- Alginate
- Agarose
- HEMA –MMA
- Cellulose sulfate
- Chitosan
- Acetate

Configuration
- Mixture cells + polymer
- Droplets (extrusion method)
- Gelification
- Membrane

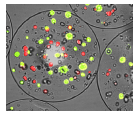
Characteristics of the membrane
- Membrane permeability
- Stability
- Biocompatibility
- Efficient mass transfer
- Mechanical properties

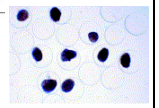
Cell microencapsulation purpose



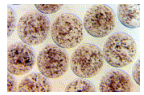
Orive *et al.*, Trends in Biotechnology, vol. 24, No. 5, 2003

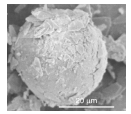
Cell microencapsulation in biomedical field





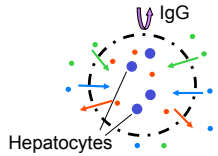
Cell type	Application
Fibroblasts	Metabolic deficiencies, neurotrophic factors, epilepsy
Myoblasts	Metabolic deficiencies, neurotrophic factors, cancer
Kidney cells	Hemophilia, neurotrophic factors, antiangiogenesis
Pancreatic islets	Diabetes
Ovary cells	Fabry disease
Parathyroid cells	Artificial organs
Hepatocytes	Liver transplantation
Chondrocytes	Bone and cartilage regeneration
Leydig cells	Hormone replacement
Adrenal chromaffin cells	Parkinson's disease, chronic pain
Stem cells	Bone regeneration
PC12 pheochromocytoma cells	Neurotrophic factors, neurotransmitters
Myeloma cells	Hepatic growth factor
Hybridoma cells	Antibody production
Tumor cells	Cancer vaccine, interleukins
Virus producer cells	Cancer
Bacteria	Elimination of urea





Orive *et al.*, Trends in Biotechnology, vol. 22, No. 2, 2004

Hepatocytes microencapsulation

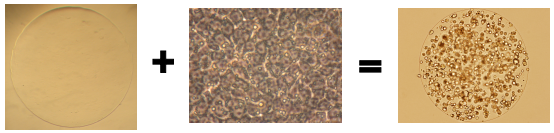


Nutrients

Oxygen

Metabolites

Alginate bead



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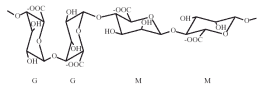
Alginate bead choice

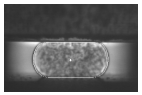
Alginate properties :
- Diffusion
- Mechanical resistance
- 3D Environment



Mooney *et al.*, Biomaterials, 25, 2004

Structure :
- Polysaccharide
- Gellification (Ca^{2+}, Br^{2+}...)

Important parameters which have an influence on diffusive and mechanical properties :
- Gellification time
- Ratio M/G
- Alginate concentration



Legallais *et al.*, International Journal of Artificial Organs, 2006

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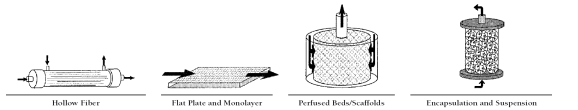
Different systems for treating hepatic diseases

Artificial liver :
- Detoxication functions

Bioartificial liver :
- Detoxication functions
- Metabolic activities (proteins synthesis...)



Bhatia *et al.*, Cell and Developmental Biology, 13, 2002

Type of cells: primary cells, immortalized cell line
Origin : human, pig
Condition : Isolated, aggregates

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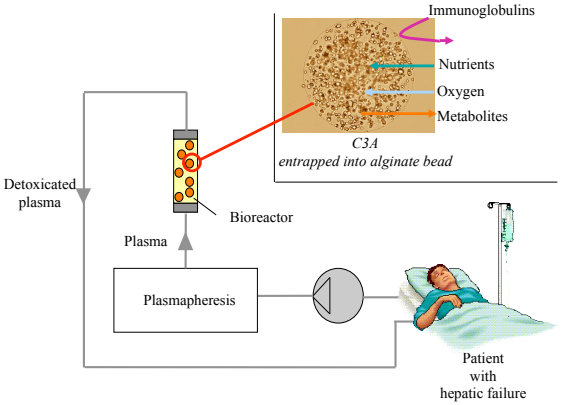
BAL : Fluidized bed bioreactor



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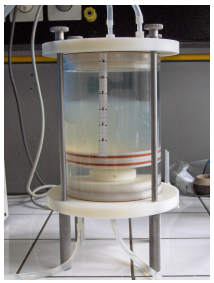
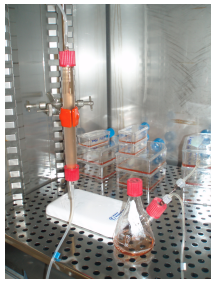
Different scale for bioreactor

Human scale



Height : 45 cm
Internal diameter : 5,4 cm

Scale for experiments



Height : 17 cm
Internal diameter: 1,35 cm

Study purposes

Mass transfer in the Bioartificial Liver

- diffusive in the bead itself
- diffusive + convective in the supernatant

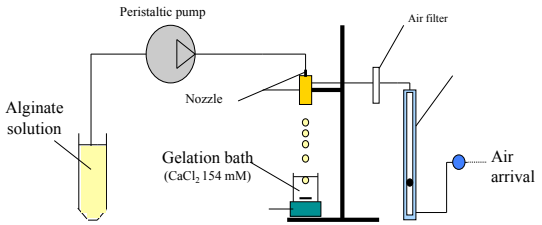
Absence of objective data :
-on the optimum size of beads
- on the optimum viscosity/type of alginate
in the fluidised bed bioartificial liver

Influence of beads diameter (600 and 1000 µm)

Influence of different types of alginate

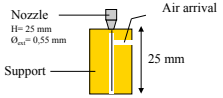
- Biological behaviour of encapsulated cells (C3A)
- Vitamin B12 marker

Microencapsulation process



Different type/viscosity of alginate:

Alginate low viscosity (2.2%)
Alginate medium viscosity (1,5%)



Different diameter of beads:

1000 µm
600 µm

Cell behaviour study

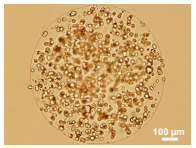
Cell line **C3A** from ATCC

Medium culture

- MEM Earles salts (MEM), L-glutamine
- penicillin (100 units/ml) - streptomycin (100 µg/ml)
- 1 % of non-essential Amino Acids
- 1 % of Hepes buffer solution 1 M
- 1 % of sodium pyruvate 100 mM
- 10 % of fetal calf serum (FCS)

Functions

ammonia synthesis
glucose consumption
albumin synthesis
AFP secretion



C3A in alginate bead
1000 µm

Cell number per bead

~ 4500 cells / bead 600 µm
~ 21 000 cells / bead 1000 µm

Mass transfer study

Solute	Molecular weight (Da)	Presence of cells
Ammonia	17	Yes
Glucose	180	Yes
Vitamin B12	1355	No
Albumin	60 000	Yes
Alpha-foetoprotein	70 000	Yes

ATCC comments:
C3A is clonal derivative of Hep G2 that was selected for strong contact inhibition of growth, **high albumin production, high production of alpha fetoprotein (AFP)** and ability to grow in glucose deficient medium. As the cells become confluent, there is a marked reduction in AFP secretion and an increase in albumin secretion. Gluconeogenesis activity is strongly oxygen dependent.

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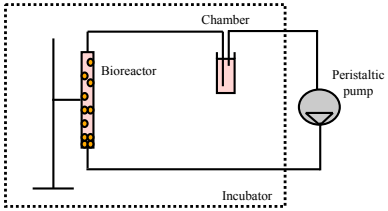
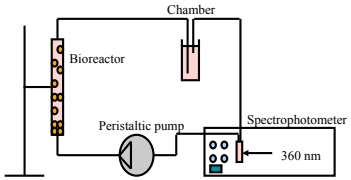
Experimental set ups

CELL CULTURE
24 hours



MASS TRANSFER
with Vitamin B12



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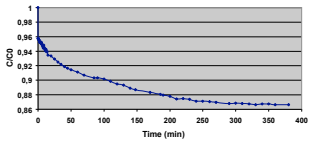
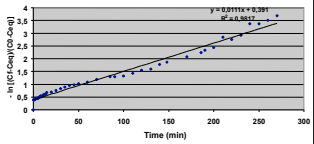
Determination of transfer coefficient K

Vitamin B12 Absorption



Transfer coefficient calculation K



Theoretical model (Fick's law)

$$\frac{d(V_f C_f)}{dt} = -\frac{d(V_a C_b)}{dt} = -KA\,(C_f - C_b)$$

K : transfer coefficient
A : total surface of the beads
V_f : fluid volume
V_a : accessible volume for the Vit. B12

$$C_f(t) = C_{eq} + (C_0 - C_{eq}) \exp\left[-\frac{KA}{V_a V_f}(V_a + V_f)\,t\right]$$

$$-\ln\left[\frac{C_f - C_{eq}}{C_0 - C_{eq}}\right] = \frac{KA}{V_a V_f}(V_a + V_f)\,t$$

Slope

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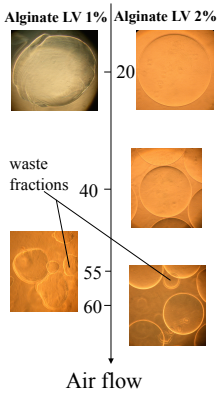
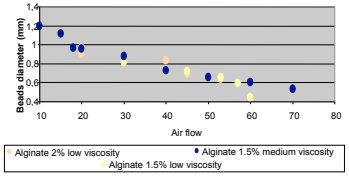
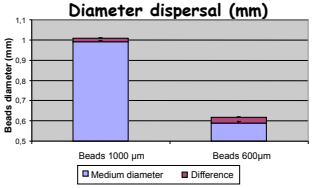
Optimization of bead production



Effect of air flow on beads diameter



Diameter dispersal (mm)



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Cell viability inside alginate bead

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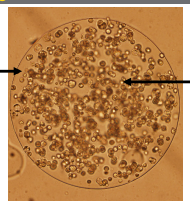
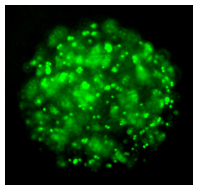
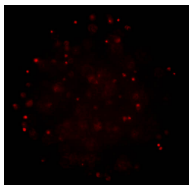


Orange Acridine



Propidium Iodide



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Effect of bead diameter on ammonia synthesis

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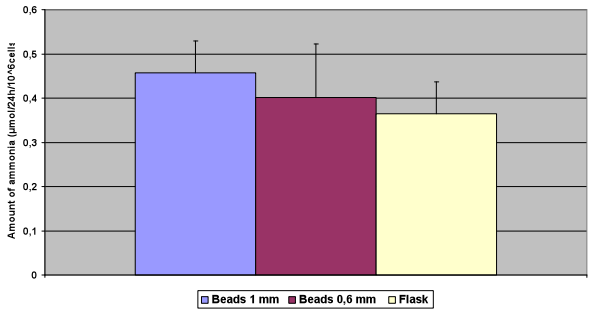

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Effect of alginate viscosity on ammonia synthesis

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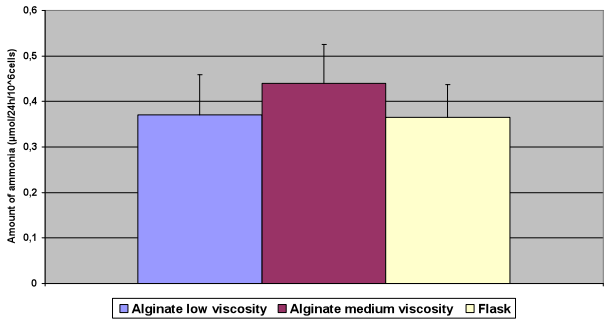

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Effect of bead diameter on glucose consumption

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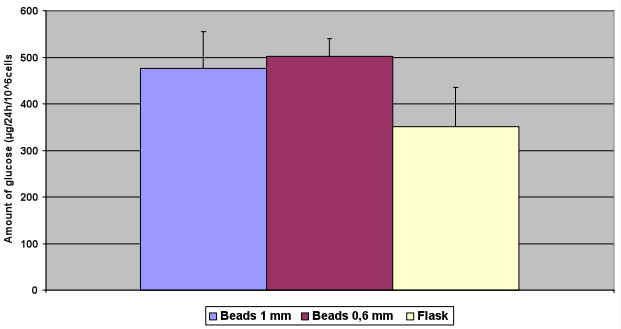

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Effect of alginate viscosity on glucose synthesis

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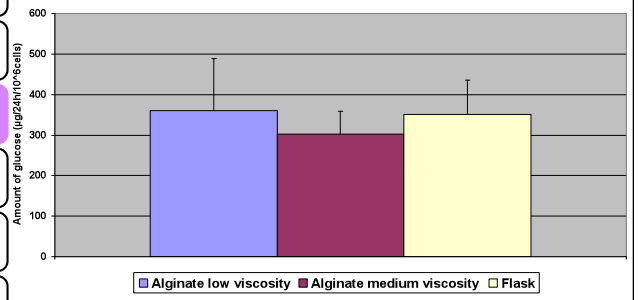

Effect of bead diameter on albumin synthesis

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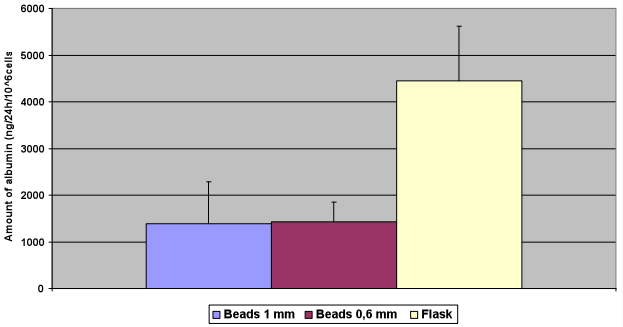

Effect of alginate viscosity on albumin synthesis

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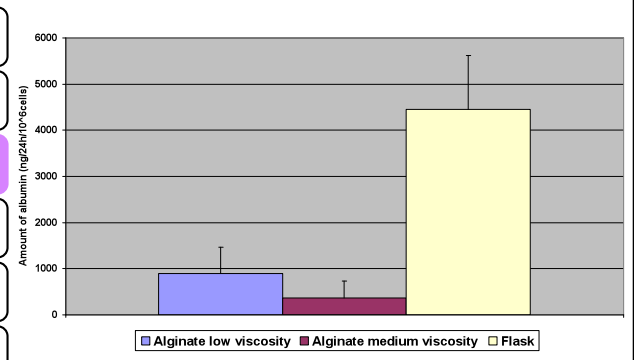

Effect of bead diameter on AFP synthesis

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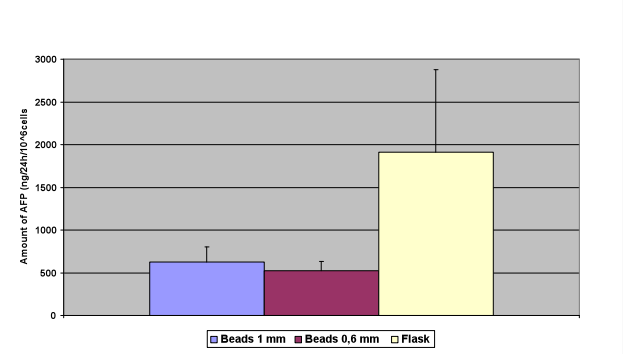
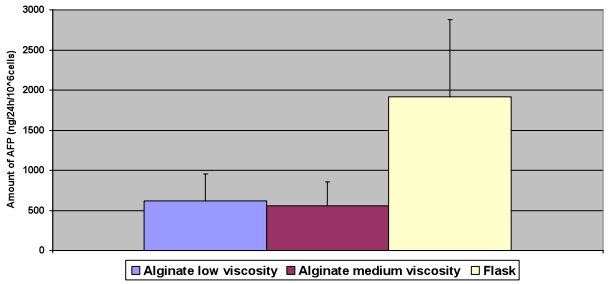
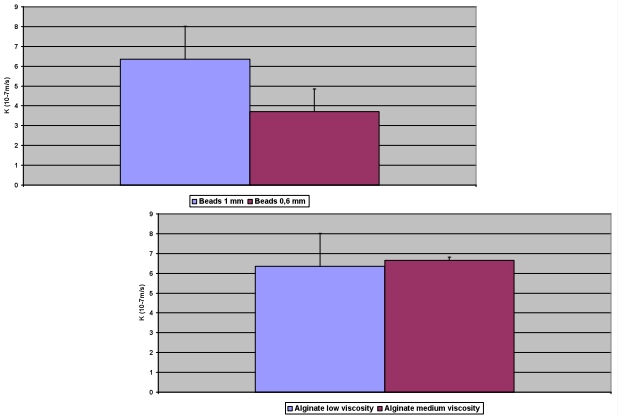


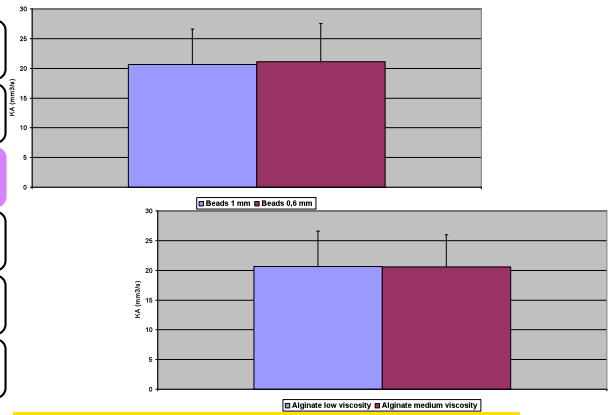
Effect of alginate viscosity on AFP synthesis



Mass transfer coefficient K



Mass transfer coefficient K corrected with bead surface



- **Biological results** are **similar** :
  - for **beads 600** and **1000 µm**
  - for **low** and **medium alginate viscosity**

The diameter of microbeads and the properties of alginate does not influence the synthetic function of hepatic cells

- **Results conforted** by **mass transfer** with the **Vitamin B12**

No effect :

To favour the production of beads 1000 µm, easier to make and best quality

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Optimize geometries of beads/capsules for optimum mass transfer

Choose : - the best biomaterial
- the best type of gelification solution
- the best concentration of gelification solution
- the best time of gelification

Identify the best microencapsulation method

Immunoprotection has to be ensured with or without membrane

Control the cut off of the membrane

Use others markers to check mass transfers without cells

Use mechanical fluids tools to model mass transfer

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