

UNIVERSITÉ DE TECHNOLOGIE
COMPIÈGNE

BIOLOGICAL ENGINEERING

UMR CNRS 6600 Biomechanics and BioEngineering

**Hepatocytes microencapsulation
for medical application :
Bioartificial Liver**

A. Gautier, M. Dufresne, Q. Vu Dinh, B. Carpentier,
P. Paullier, C. Legallais

CNRS

Biocapsulation Research Group

utc

LE CONSEIL RÉGIONAL DE PICARDIE

Microencapsulation parameters 2 / 30

Backgrounds

Material

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

Methods

Results

Conclusions

Prospects

Welcome to UTC

Configuration

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

Characteristics of the membrane

- Membrane permeability
- Stability
- Biocompatibility
- Efficient mass transfer
- Mechanical properties

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Cell microencapsulation purpose 3 / 30

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Inward diffusion of nutrients and oxygen is allowed

Antibodies are excluded

Inflammatory cells are excluded

Therapeutic and waste products can diffuse freely

Therapeutic product-secreting cells

Microcapsule

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

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Cell microencapsulation in biomedical field 4 / 30

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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

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Alginate bead choice

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Alginate properties :

- Diffusion
- Mechanical resistance
- 3D Environment

The diagram illustrates the repeating unit structure of alginate. It shows two types of units: G (gamma-D-glucuronate) and M (alpha-L-guluronate). The G unit has a carboxylate group (-COO-) at the C4 position and a hydroxyl group (-OH) at the C5 position. The M unit has a hydroxyl group (-OH) at the C4 position and a carboxylate group (-COO-) at the C5 position. The linkage between the units is a beta-1,4-glycosidic bond.

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

A black and white micrograph showing a cross-section of alginate beads. The beads appear as small, irregularly shaped structures with a porous or hollow interior, likely representing microcapsules used for hepatocyte encapsulation.

Legallais et al., International Journal of Artificial Organs, 2000

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aude.gautier@utc.fr

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Different systems for treating hepatic diseases

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Artificial liver :
Detoxification functions

Bioartificial liver :
Detoxification functions
Metabolic activities (proteins synthesis...)

The diagram shows four schematic representations of liver support systems:

- Hollow Fiber:** A central tube with arrows indicating flow, surrounded by a porous membrane.
- Flat Plate and Monolayer:** A grid-like structure with arrows indicating flow across the surface.
- Perfused Beds/Scaffolds:** A cylindrical structure with a central tube and a porous outer layer.
- Encapsulation and Suspension:** A cylindrical structure containing numerous small, irregularly shaped particles.

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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The diagram illustrates the BAL (Biosartificial Liver) system. On the left, a vertical stack of buttons lists the project's sections: **Backgrounds**, **Methods**, **Results**, **Conclusions**, **Prospects**, and **Welcome to UTC**. The main central part shows a schematic of the bioreactor setup. Detoxified plasma enters from the top left, passes through a filter labeled "Bioreactor", and then enters a "Plasmapheresis" unit. The output of the plasmapheresis unit goes to a patient lying in a hospital bed, labeled "Patient with hepatic failure". A magnified inset on the right shows an alginate bead containing hepatocytes (represented by orange circles). Labels indicate that the bead contains "C3A entrapped into alginate bead", "Immunoglobulins", "Nutrients", "Oxygen", and "Metabolites".

Different scale for bioreactor 9 / 30

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Human scale

Height : 45 cm
Internal diameter : 5,4 cm

Scale for experiments

Height : 17 cm
Internal diameter: 1,35 cm

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Study purposes 10 / 30

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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

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Microencapsulation process 11 / 30

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Peristaltic pump

Different type/viscosity of alginate:
Alginate low viscosity (2,2%)
Alginate medium viscosity (1,5%)

Different diameter of beads:
1000 µm
600 µm

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Cell behaviour study 12 / 30

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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

Cell number per bead
~ 4500 cells / bead 600 µm
~ 21 000 cells / bead 1000 µm

C3A in alginate bead 1000 µm

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Mass transfer study

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Solute	Molecular weight (Da)	Presence of cells
Ammonia	17	Yes
Glucose	180	Yes
Vitamin B12	1355	No
Albumin	60 000	Yes
Alpha-fetoprotein	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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aude.gautier@utc.fr

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Experimental set ups

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CELL CULTURE
24 hours

MASS TRANSFER with Vitamin B12

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Determination of transfer coefficient K

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Vitamin B12 Absorption

Theoretical model (Fick's law)

$$\frac{d(V_t C_t)}{dt} = - \frac{d(V_b C_b)}{dt} = - K A (C_t - C_b)$$

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

$$C_t(t) = C_{eq} + (C_b - C_{eq}) \exp \left[- \frac{KA}{V_t V_b} (V_b + V_t) t \right]$$

$$- \ln \left[\frac{C_t - C_{eq}}{C_b - C_{eq}} \right] = \frac{KA}{V_t V_b} (V_b + V_t) t$$

Slope

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Optimization of bead production

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Effect of air flow on beads diameter

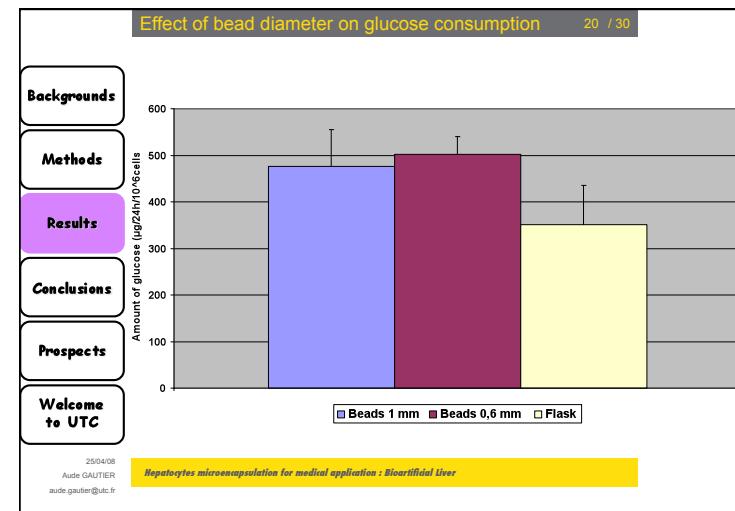
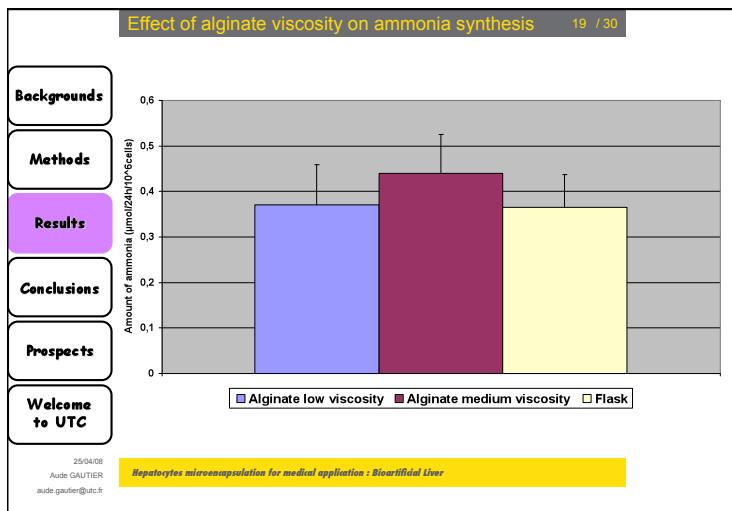
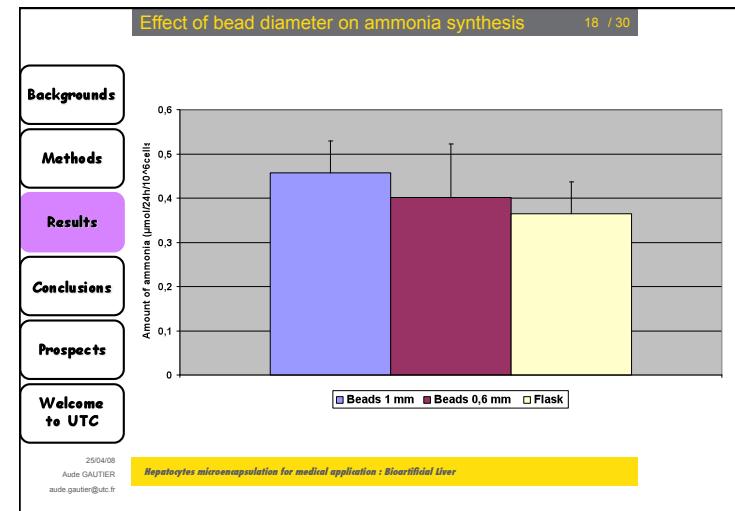
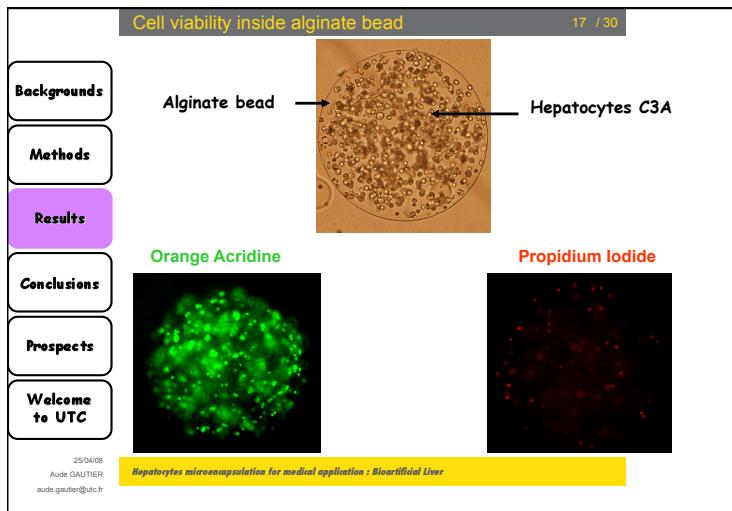
Alginates 1% low viscosity
Alginates 1.5% medium viscosity

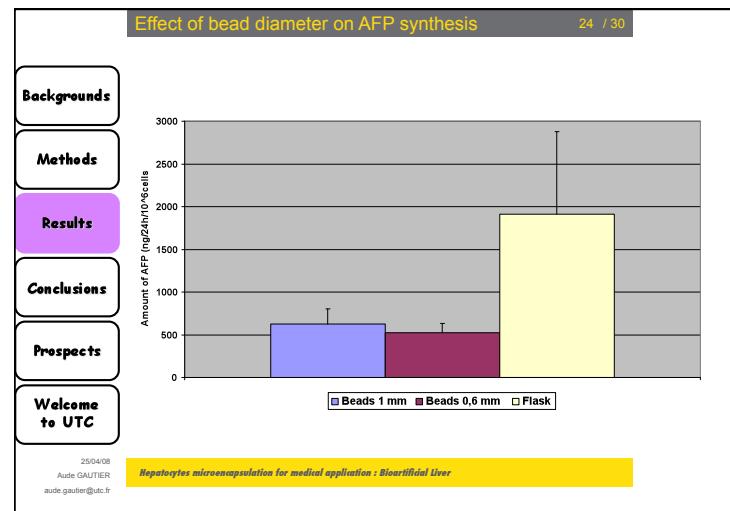
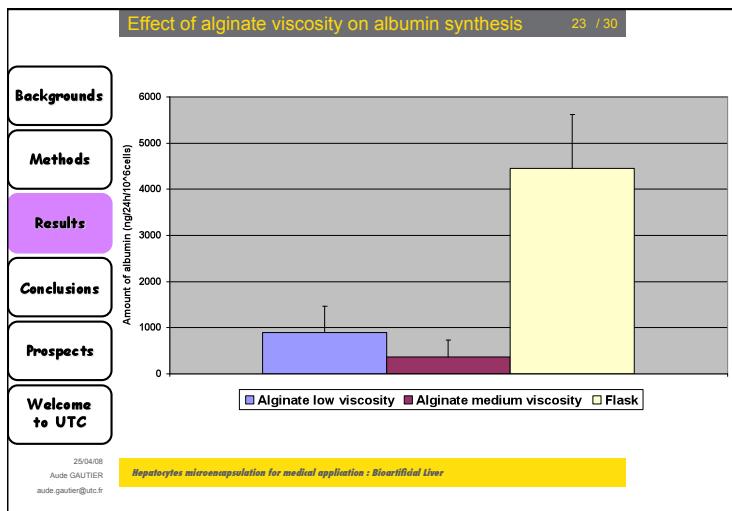
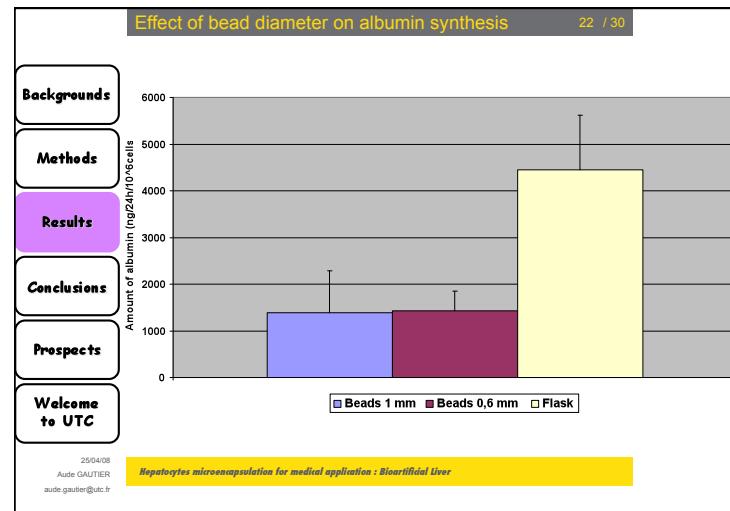
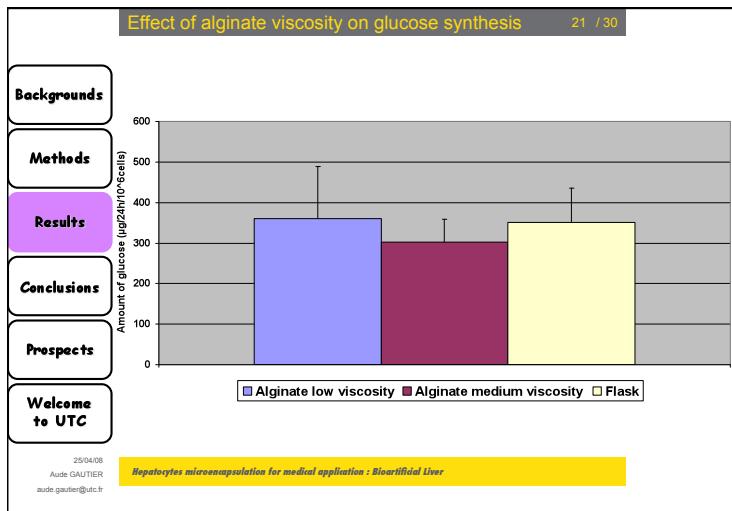
Diameter dispersal (mm)

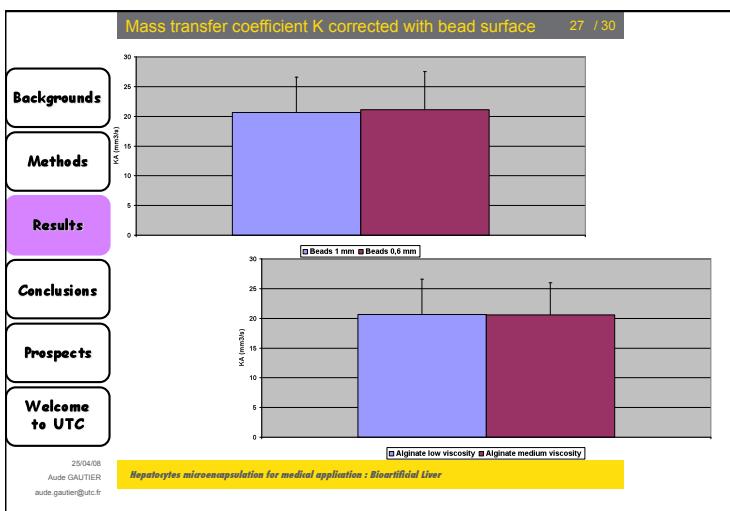
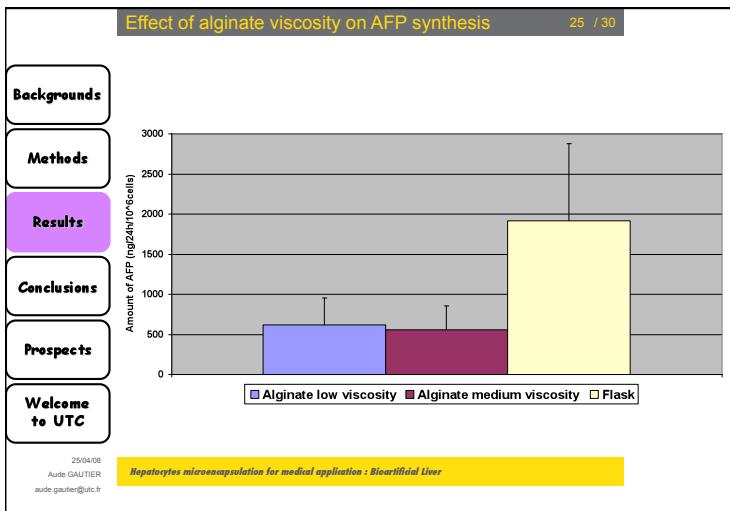
Beads 1000 μm
Beads 600μm
Medium diameter
Difference

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- Biological results are similar :
 - o for beads 600 and 1000 μm
 - o 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 confor ted 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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Backgrounds

Optimize geometries of beads/capsules for optimum mass transfer

Methods

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

Results

Identify the best microencapsulation method

Conclusions

Immunoprotection has to be ensured with or without membrane

Prospects

Control the cut off of the membrane

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- Use others markers to check mass transfers without cells
- Use mechanical fluids tools to model mass transfer

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aude.gautier@utc.fr