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PRILLING PROCESS APPLIED TO COLLAGEN SOLUTIONS

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SUMMARY

The present work concerns the preparation of microbeads of collagen by a prilling process.

Collagen is one of the main components of vertebrate proteins, especially in such tissues as skin, tendons, placenta, ... Furthermore, it has some properties which makes it interesting to use as a biomaterial : biocompatibility, biodegradability, high tensile strength, hemostatic power, participation to the wound healing.

The patented process which is developped in this paper combines two techniques :

- breaking of a capillary flow by prilling

and

- reticulation of collagen after oxydation with periodic acid.

It uses neither organic solvents, nor variation of temperature and allows the production of microbeads which can be utilized for many different medical applications.

PRILLING PROCESS

APPLIED TO COLLAGEN SOLUTIONS

INTRODUCTION

Collagen, a glycoproteic fibrous macromolecule, is the main component of the extracellular matrix of conjunctival tissues in vertebrates [1-2].

Whether extracted from animals hides, tendons or human placenta, collagen is used for various biomaterials because of its physical, chemical and biological properties (high resistance to stretching, weak extensibility, possibilities of reticulation, weak antigenicity, ability to agregate platelets, participation in cellular growth) [3-4].

Human placental collagen, which was used in this experiment, allows the preparation of solutions, gels, films, fiber solids (sponges, tissues, micro-particles, capillaries, guts, suture thread) in which viral sterility is ensured due to the method of preparation for these collagens [5].

Type IV collagen obtained from human placenta is already used in some surgical biomaterials either as viscoplastic solution in ocular surgery [6] or as corneal implant [7].

The particulate form of collagen can have many applications : micro-carriers of active substances, drug delivery systems, micro-support of cellular culture, hemostatic powder, ...

Collagen particles can be obtained by different methods. Some of these processes denature the glycoprotein due to the high temperatures required ; other methods are dangerous or expensive due to a complex process because of the necessity of using organic solvents to obtain emulsions.

Prilling of microparticles of collagen seems to be a process of high interest ; it is simple, flexible and gives a large productibility ratio.

These attributes explain our interest to develop a system based on prilling.

PRILLING PROCESS

Prilling has already been used for a long time in the chemical industry (metals, waxes, fertilizers) to obtain beads of 0.8 to 3 mm diameter. In these specific applications, the product is melted (by application of heat) or used as a concentrated suspension ; it is ejected out of a nozzle with many holes. The spray breaks out and prills are formed by crystallizing during the cooling process while falling before becoming solid.

The falling medium is often gas : prills fall down in air or helium contained in a prilling tower several meters high. The medium can also be liquid : water, liquid nitrogen, etc....

Industrial pharmacy also uses prilling to make capsules of pharmaceutical molecules.

To adjust prilling to collagen without thermic denaturation, we used the reticulation process based on oxydation with periodic acid which allows to polymerisation of collagen by changing the pH [8].

THE APPLICATION OF PRILLING TO COLLAGEN

1. Material (diagram 1)

A storage container (1) with a precision manometer (2) is connected to a discharge of compressed air which permits the induction of the liquid to the hollow needle (3) after passing through a porous filter (membrane).

A generator of frequency 10 Hz - 1 MHz (4) coordinated with an amplifier (5), transmits through a vibrator (6) a modular horizontal periodic movement to the discharged needle. The needles we used have an interior diameter varying from 0.2 to 0.6 mm.

Observed with a stroboscope (7), falling prills can be seen to have homogenous spherical shapes, each equally distant from the other.

Finally, prills are collected in a becher (8) containing the buffer solution, which is stirred by a magnetic agitator (9).

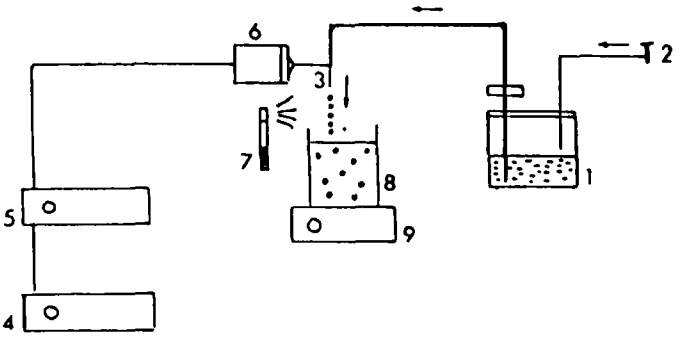

Diagram 1:

Material of prilling process

2. Methods

2.1. *Preparation of collagen solutions*

We used type III+I human collagen but type I bovine collagen can also be used.

An acid solution of type III+I collagen is first prepared and then oxydized by periodic acid. Oxydation generates several aldehydic functions on the mono- and disaccharide units and on the hydrolysine of collagen molecule. At neutral or alcaline pH, these groups can react with amino groups of the same molecule or of another molecule of collagen [8].

In this manner, collagen is reticulated.

2.1. *Processing of Prilling (diagram 2)*

The collagen solution to be prilled flows at a constant output and in a laminar flow through a hollow needle of small diameter. These conditions can be met through the application of a constant air presure on the storage container. A continuous stream is ejected out of the needle without breaking. An horizontal vibration is then applied to the needle with a calculated frequency which depends on the liquid. The continuous stream is cut in equal sized sticks which rapidly acquire a spherical form due to the effect of interfacial gas-liquid tension.

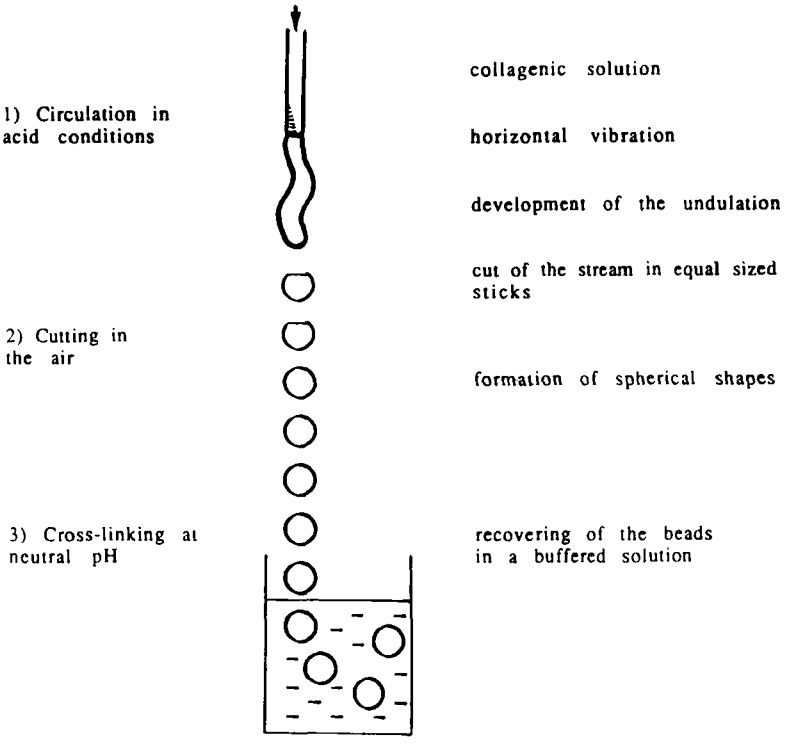

Diagram 2:

Processing of prilling

The efficient frequency of vibration is determined by observation with a stroboscope (figure 1). The prills should appear as a string of homogeneous beads. These beads are recovered in a buffered solution which induces their immediate polymerization. To avoid overlapping of prills, the solution is pushed at a linear speed which is higher than the falling speed of the beads.

Reticulation occurs by neutralizing the pH.

Beads are collected by sifting or centrifugation and then washed with distilled water or buffered solution to eliminate reactive agents and salts which could possibly still be present as contaminants.

The final product is a suspension which is more or less concentrated depending on future use.

PRILLING : FUNDAMENTAL ASPECTS

The main parameters of this particular technique of prilling applied to collagen solutions can be defined by different theories of hydrodynamics.

We must consider :

- optimal vibration of discharge needle,
- free fall velocity of prills in the air,

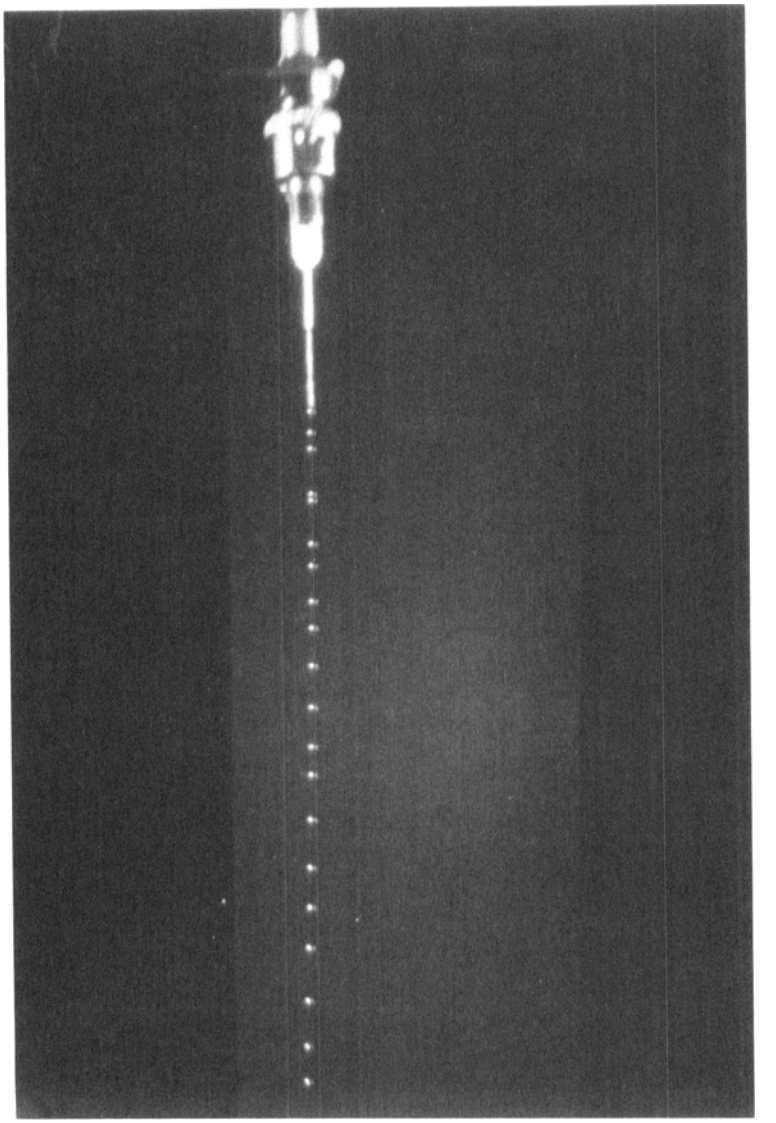

Figure 1:

Picture of the prilling process seen with the stroboscope.

- hydrodynamics of circulating fluid,
- number of prills formed and production rate.

1. Optimal vibration of needle

One can observe that there is a certain frequency at which the production of prills does not become random. At this frequency, called "efficient frequency", the prills are isolated in the air as a very regular string of beads.

The wavelength of this efficient frequency can be determined by the RAYLEIGH-WEBER law (equation 1)

$$\lambda = 4{,}443\, d \sqrt{\frac{3\mu}{\rho\, \sigma\, d} + 1}$$

λ	=	efficient wavelength (nm)
d	=	diameter of the hole (m)
μ	=	dynamic viscosity ($kg.m^{-1}.s^{-1}$)
ρ	=	specific weight of liquid ($kg.m^{-3}$)
σ	=	liquid interfacial tension between liquid and air ($kg.s^{-1}$)

Equation 1 shows that the wavelength :

a) depends much more on aperture diameter "d" and on the solution viscosity "μ" than on the apparent density and interfacial strain, both of which are in the forth root in the equation ;

b) is independent of the hydrodynamics of the circulating liquid and consequently independent of the pressure applied to the liquid.

2. Calculation of the terminal velocity of falling prills in the air

The terminal velocity of falling prills in the air can be determined by using the efficient frequency of breaking of the solution.

The following calculation shows that the hypothesis of a viscous flow around the falling prills is not acceptable and the drag force must be taken into account.

a) If we postulate that the flow of air around each falling prill is purely viscous, the falling rate can be given by STOKES law :

$$Vlim = \frac{(\rho_s - \rho_a)\, d^2}{18\, \mu_a}$$

with, in the case of collagen solution :

ρ_s	=	density of the collagen solution	=	1000 $kg.m^{-3}$
ρ_a	=	density of the air	=	1.3 $kg.m^{-3}$

d	=	diameter of one bead	=	10^{-3} m
g	=	gravitational acceleration	=	9.8 $m.s^{-2}$
μ_a	=	viscosity of the air	=	$18.10^{-6} kg.m^{-1}.s^{-1}$

we obtain : V = 30 $m.s^{-1}$

The Reynolds number for falling prills in the air is given by the equation :

$$Re = \rho_a \frac{V_{lim}\, d}{\mu_a}$$

Here, we obtain : Re = 2100

This Reynolds number is higher than the accepted initial values for the flow rate of purely viscous solutions. This value is Re = 0,2. A hypothesis for a viscous flow-rate cannot be formulated.

b) According to the speed calculated using the above equation, it is necessary to take into account the drag force coefficient of the prill : Cx which is a decreasing function of Re.

Through successive approaches and for the pair Re = 200 and Cx = 1,4, the equation which gives the terminal velocity is :

$$V_{lim} = \sqrt{\frac{4 d \rho_s g}{3 Cx \rho_a}}$$

Numerical example : V_{lim} = 2.7 $m.s^{-1}$

and Re = 195 which is a value of Reynolds close enough to the hypothesis value.

Conclusion : One bead of 1 mm diameter obtained under conditions described above has a terminal velocity of fall of 2.7 $m.s^{-1}$.

3. Hydrodynamics of circulating fluid

In order to efficiently separate the viscous solution into microspheres it is important to know how the collagen solution flows down in the capillary tube. This flow must be laminar.

It is characterized by Reynolds number which in the case of circulation in a tube can be expressed as follow :

$$Re = \rho_s \frac{U.D}{\mu}$$

The flow is laminar if Re < 2000.

U	=	velocity of flow	=	1.2 $m.s^{-1}$
ρ_s	=	density of the collagen solution	=	1000 $kg.m^{-3}$

D	=	diameter of the hole	=	4.10^{-4} m
μ	=	viscosity of the solution	=	0.8 $kg.m^{-1}.s^{-1}$

in this case : $\mathcal{R}e = 0.6$.

The Reynolds number calculated in this equation is less than 2000 ; this means that the solution of collagen has a laminar flow in the output needle.

N.B. : - The average velocity of flow is calculated for a flow rate of 9 ml/min. and an inside needle diameter of 0.4 mm.

- Viscosity of collagen solution used in the calculation above is determined with a coaxial cylinder rheometer.

4. Measure of the production of prills using a stroboscope

The stroboscope used in the prilling process to observe the beads can also be used as a drop counter to measure the flow rate of the system.

Production rate of the beads can be calculated using 2 frequences of the stroboscope (f and f'). At such frequencies, the stroboscope show n and n' immobile beads. The beads fall on a distance l and l' with a speed v during the times t and t'.

Then, if

d	=	distance between 2 successive beads
l	=	distance between n successive beads
v	=	falling speed of 1 bead
t	=	falling time for 1 bead in a distance l

we can write down for 2 lengths l and l' :

$$l = v.t = n.d$$
$$l' = v.t' = n'.d$$

$$\Rightarrow t-t' = (n-n')\ \frac{d}{v}$$

with : $n = n' + 1$

$$t-t' = \frac{d}{v}$$

$$\frac{1}{f} - \frac{1}{f'} = \frac{d}{v}$$

$$\frac{d}{v} = \frac{f'-f}{f.f'}$$

$$\Rightarrow \quad \frac{v}{d} = \frac{f.f'}{f'-f}$$

with $\frac{v}{d}$ = productivity of system in beads per minute.

◊ *Application* :

For example, for a flow rate of d = 9 ml/min. we obtained with the stroboscope :

- 6 apparently immobile drops for 4350 $t.min.^{-1}$
- 7 apparently immobile drops for 3750 $t.min.^{-1}$

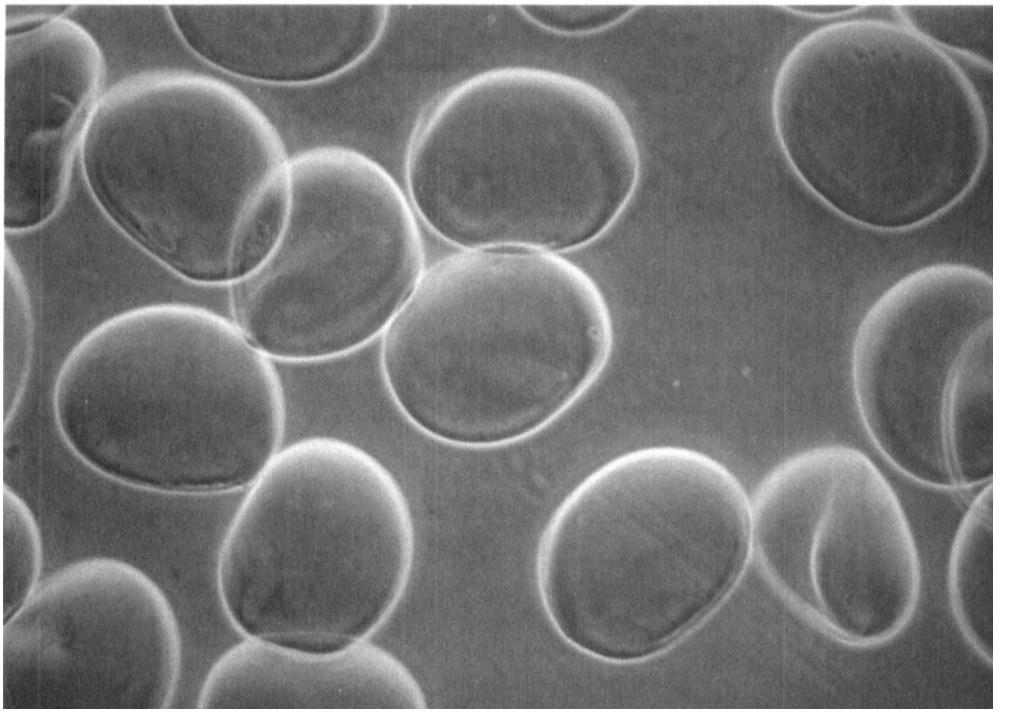

Figure 2:

Morphological aspect of particles obtained with a needle of interior diameter of 0.2 mm.

According to the formula : number of drops per time $= \frac{f.f'}{f'-f}$

Numerical example : flow rate = 20 000 drops/min.

This calculation is verified by measuring the volume of liquid flowing during a given period of time and by estimating the diameter of one drop.

5. Properties of beads obtained by prilling

5.1. *Morphological aspect (figures 2 and 3)*

Table 1 gives the two perpendicular diameters of the particles obtained by prilling with two types of needle (interior diameter 0.2 mm and 0.4 mm).

The medium diameters measured are about three times larger than the anterior diameter of each needle.

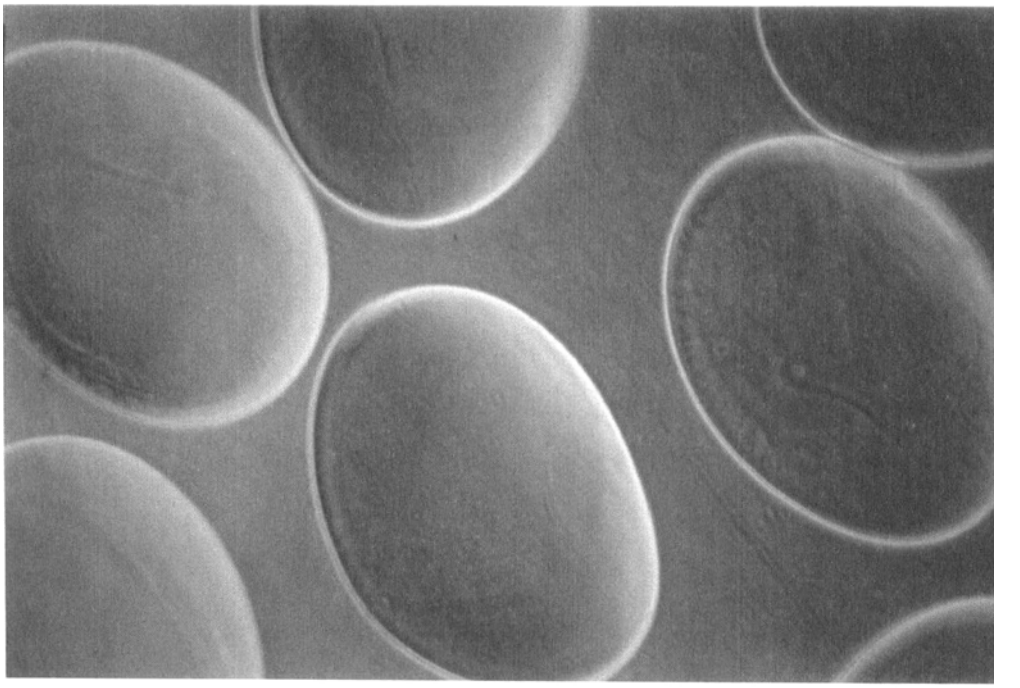

Figure 3:

Morphological aspect of particles obtained with a needle of interior diameter of 0.4 mm.

TABLE 1

PARTICLES 	INTERIOR DIAMETER OF THE NEEDLE	
	0,2 mm	0,4 mm
d1 (mm)	0,51 ± 0,04	0,9 ± 0,01
d2 (mm)	0,62 ± 0,03	1,32 ± 0,06

TABLE 2

ANISOTROPY	INTERIOR DIAMETER OF THE NEEDLE	
	0,2 mm	0,4 mm
d2/d1 ≤ 1,2	64%	4%
1,2 < d2/d1 ≤ 1,5	34%	66%
d2/d1 > 1,5	2%	30%

5.2. *Granulometric distribution*

Table 2 gives the granulometry of each particle studied.

For the 0.2 mm needle : 64 % of the particles are spherical ($d_2/d_1 \leq 1.2$) ; 34 % are spheroïdal ($1.2 < d_2/d_1 \leq 1.5$) and 2 % are disformed in their length.

We can notice that for a diameter of 0,2 mm the percentage of spherical beads is the most important.

5.3. _____

Figure 4 and 5 show the surface of the particles. A fibrillar matrix of collagen is observed with micropores of about 0.1 µm.

Figure 6 shows a microbead of type III+I collagen covered with an epidermal cell monolayer.

Human keratinocytes were cultivated statically, without stirring.

Attachment and proliferation of such cells are satisfying, demonstrating that the collagen microbeads made by prilling process are not cytotoxic and represent a good substrate for keratinocytes.

CONCLUSION

The fabrication of humid particles from collagen solutions can be obtained by the process of prilling. This process uses neither organic solvents, nor variation of temperature. Therefore, it does not denature the collagen molecule. The reticulation occurs simply by changing the pH without chemically altering the molecule.

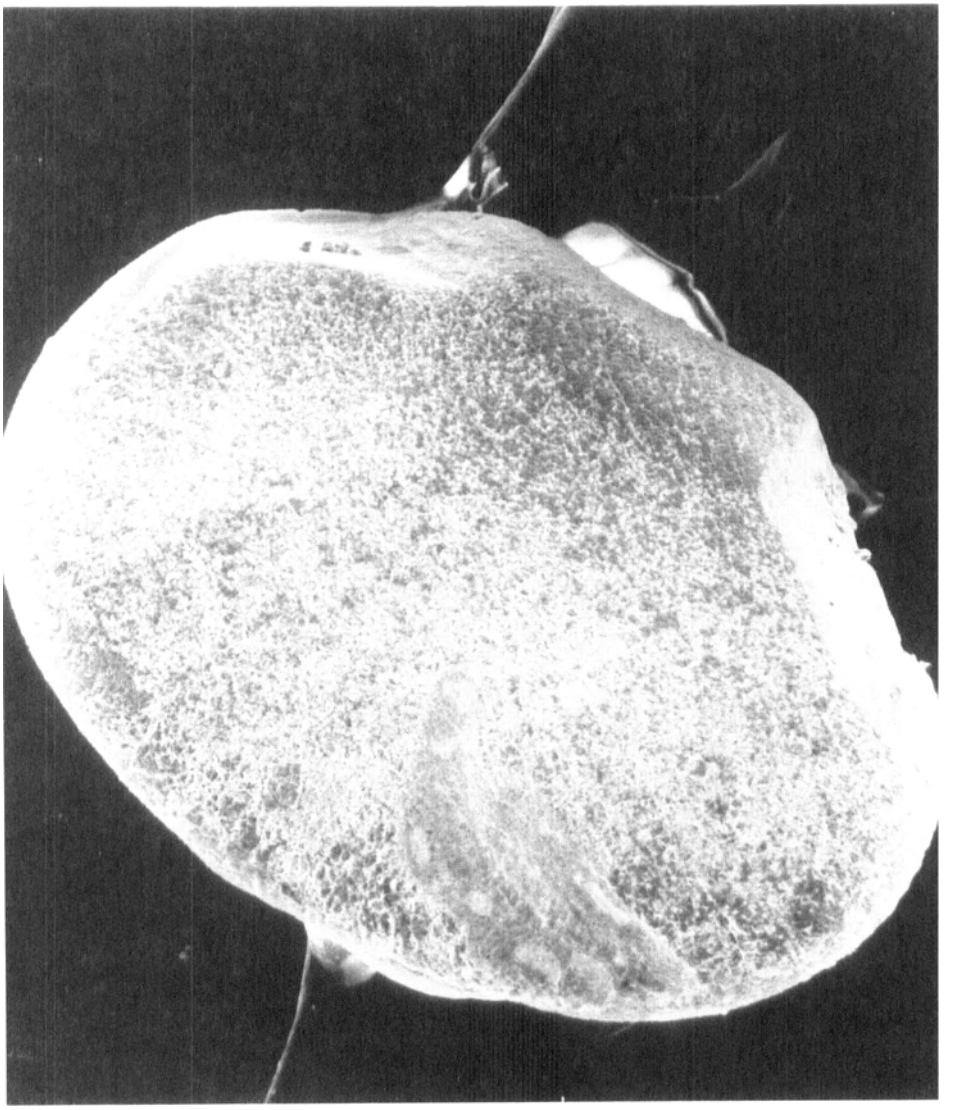

Figure 4:

Surface aspect of a particle (x 340), electron microscope photography.

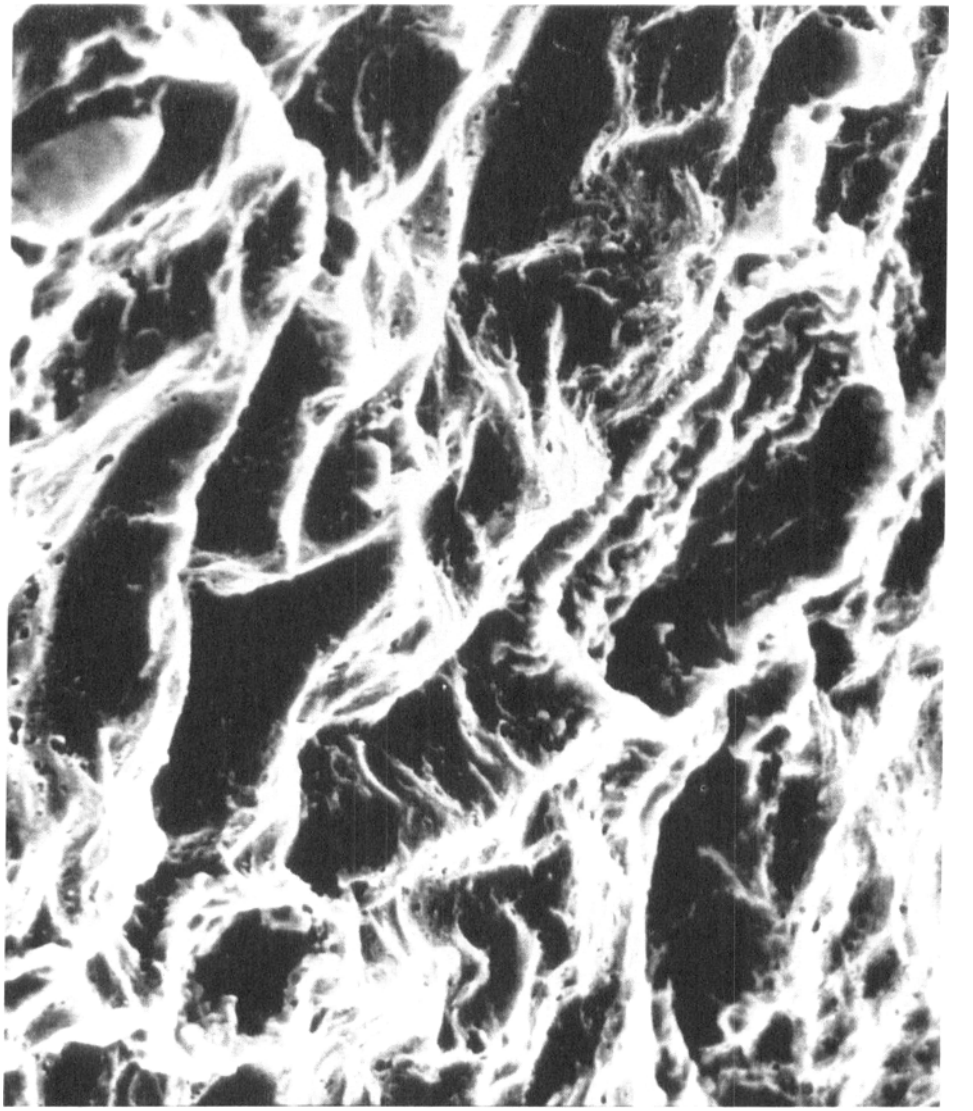

Figure 5:

Surface aspect of a particle (x 30000), electron microscope photography.

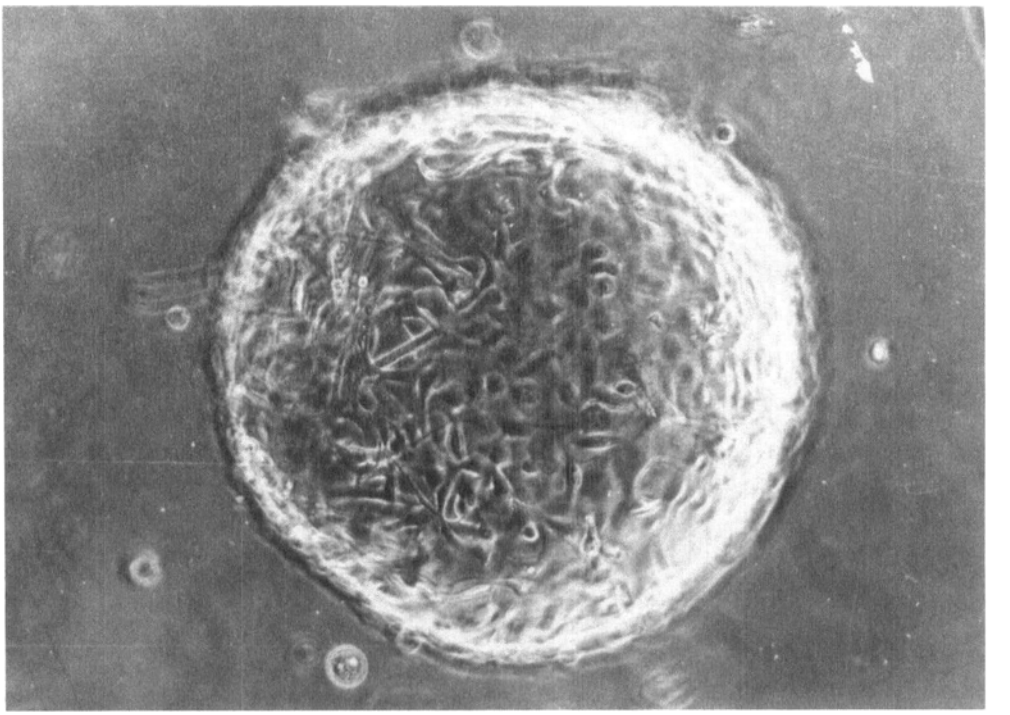

Figure 6:

A collagen microbead covered with a human keratinocyte monolayer obtained after 8 days in culture.

These prills are homogeneously cross-linked, biodegradable, able to combine with other molecules or cells and have no chemical toxicity (to be published).

In conclusion, these particles could be used in different applications, for example :

- drug delivery system by including active molecules in a collagen matrix during the process ;
- microcarriers for cell culture ;
- hemostatic or wound healing biomaterials.

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