**Production of multifunctional microdevices for cell transplantation by microfluidic approaches.**

**S. Mazzitelli[1], L. Capretto[1], G. Luca[2], A. Tosi[1] and C. Nastruzzi. [1] ***

[1] Department of Chemistry and Technology of Drugs and [2]Department of Internal Medicine (Di.M.I.), Section of Internal Medicine and Endocrine and Metabolic Sciences, University of Perugia, Perugia, Italy (sma@unipg.it)

## Introduction

Microfluidics is a "micro" technological field dealing with the handling of fluids, having applications in different fields, including chemical processing, food manufacturing, pharmaceutic, biotechnology and cosmetic.
In this respect, in recent years some authors have described different methods to obtain microparticles by microfluidic approaches. For instance, Takasi N. *et al*. prepared acrylic microspheres (based on poly 1,6-hexanediol diacrylate) by polimerization of a droplet suspension containing the acrylic monomer, using a T-shaped-channel microchip. These authors claimed to obtain different droplet/particle size ranging from 30 to 120 µm by varying the microdevice flow conditions.
Xu S. *et al*. reported a strategy to produce agarose monodisperse solid particles by thermally-induced gelation or liquid-solid phase transition. The flow focusing region was kept at a temperature exceeding the gelling (or solid-liquid phase transition) temperature ($T_0$). The outlet channel was cooled to a temperature below $T_0$, and the droplets solidified as they travelled down the channel. By varying the flow rates of the continuous and dispersant phase, they obtained disk whit diameters from 50 to 250 µm (S. Xu et al. 2005).
Among the polymers used for the preparation of microparticle for cell entrapment, the most commonly employed polymer is the alginate. Alginate is a biodegradable, natural polysaccharide obtained from marine brown algae. It is a linear copolymer composed of 1,4-linked d-mannuronic acid and l-guluronic acid residues. It gels in the presence of divalent cations, such as calcium and barium, due to the stacking of guluronic acid (G) blocks with the formation of “egg-box” calcium linked junctions (A. Martinsen et al. 1989). Alginate microspheres have been used for the encapsulation of a wide variety of biologically active agents, including proteins (G. Coppi et al. 2002), antibodies (M. Albarghouthi et al. 2000), DNA (D. Quong et al. 1998) and cells (G. Klinkenberg et al. 2001), due to the relatively mild crosslinking conditions of preparation.
To the best of our knowledge, the literature report only three papers that describe the use of microfluidic devices to the production of alginate beads. Amici E. *et al*. described the preparation of alginate beads by an internal gelation method (E. Amici et al. 2007). To produce alginate drops two aqueous streams, one acidic (containing D-Glucone-d-lactone) and one containing calcium carbonate suspended in a alginate solution, merge immediately prior to entering a channel where a continuous flow of sunflower oil breaks the flow of the aqueous phase to form microdroplets.
K.S. Huang *et al.* reported the use of microfluidic to elicit control over the spontaneous self-assembly of water-in-oil emulsion from a solution of Na-alginate. The mixture is then dripped into a solution containing calcium ions, resulting in the instantaneous formation of Ca-alginate microspheres. Their strategy is based on a focusing force to form narrow size distribution of self-assembling sphere structures, the so-called Na-alginate emulsion (K.S. Huang et al. 2006).
Finally, Kan Liu *et al.* described the preparation of alginate beads using a microfluidic device containing two individual flow-focusing channels and a synthesizing channel. The shape (plugs, disks, microspheres, rods or threads) and size of alginate microparticles could be tuned by adjusting the flow rates of the various streams.

In the present work we report the production of monodisperse alginate microbeads by a microfluidic system, focusing our attention especially on the gelification process.

## Materials and methods

### *Materials*

For the production of alginate microbeads were employed the following chemicals. sodium Na-alginate (IE-1105) (Inotech Biosystems International, Switzerland), barium chloride dihydrate (Riedel-de Haën, U.S.A), barium carbonate (Riedel-de Haën, U.S.A), glycerine (Comifar, Italia), sunflower oil (Collina d'oro, Italy), acetic acid (Sigma-Aldrich, Germany).

### *Methods*

In order to generate the multifasic flow necessary to the preparation of microdroplets, the microfluidic chip, "Snake mixer slide" (Thinxxs, Germany) was used. This chip is constituted of a cyclo-olefin co-polymer (COC, Topas®) and contains channels with square section of 640 µm and 320 µm.

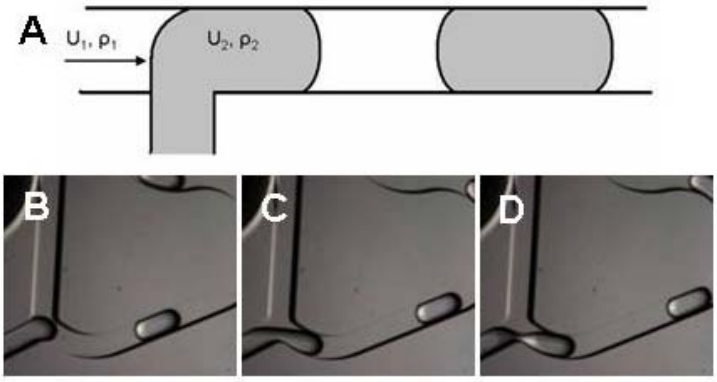


**Fig.1. Mechanism of droplet formation (squeezing). (A) Schematic representation of mechanism of squeezing (U represents the velocity of the phase and ρ represents the density of phase). Optical microphotographs of squeezing channel during the droplet formation (B-D).**

A syringe pump (KDS Model 100 Series, Kd Scientific) was employed for the injection, into the microfluidic chip, of both the dispersed and continuous phases. The microfluidic system was connected to syringes through silicone tubes. A sodium alginate solution was used as dispersed phase and slowly injected into reagent inlet of the squeezing microchannel. The second immiscible liquid (oil phase) was inject into the other inlet as continuous phase. Sodium alginate solution were forced into the oil phase at the junction of the squeezing channel to form multiphasic flow (droplets) represented from a w/o Na-alginate emulsion. Finally, the Na-alginate emulsion was gelificated by dripping, the formed w/o emulsion, into a barium (II) solution to produce Ba-alginate microbeads.

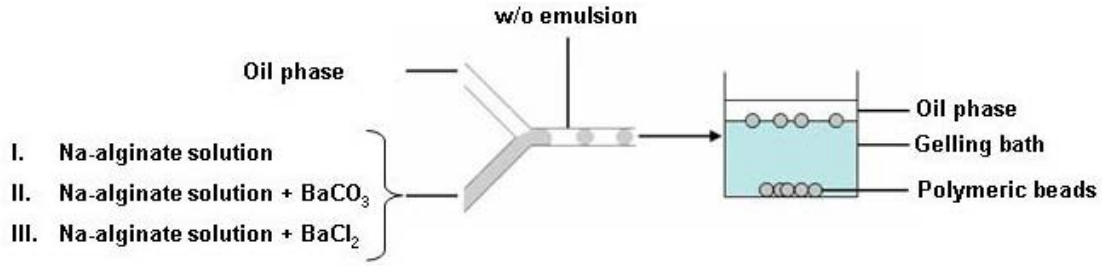


**Fig. 2. Scheme of system set up for the production of alginate beads using different dispersed phase.**

**Results**

The aim of this work was to prepare alginate microbeads with optimal morphological and dimensional characteristics in term of spherical shape and narrow size distribution.
Firstly, we produced beads by external gelation using alginate solution as dispersed phase and sunflower oil as continuous phase. After injection of the phases in the microfluidic channel, the resulting w/o emulsion was collected in a $BaCl_2$ bath to allow the gelification of the alginate droplets.
As reported in Fig. 3 A, the obtained microbeads were characterized by an elliptic shape (presence of tails). This particular shape was attributed to the slowly pass of the forming droplet through the interface between oil phase and the gelling bath. To facilitate the passage into the interface, a cyclohexane/water phase above the gelling bath was employed (Fig. 3 B) and as further improvement glycerol was added to the alginate solution, in order to increase its density (Fig. 3 C).
In both cases, there was a significant reduction of the tailed microbeads, but the elliptical shape still remained.
Successively, the internal gelation (D. Poncelet et al. 1992) procedure was assessed. To perform these experiments, acetic acid was added to the oil phase and a different content of $BaCO_3$ was suspended into the alginate solution. From the analysis of the results reported in Fig. 3 (D-F), it was evident that alginate microbeads prepared with 5.0 (D) and 10.0 mM (F) of $BaCO_3$, still presented some tails and coalescences, respectively. Only with a concentration of 7.5 mM (Fig. 3 E) we obtained beads with a regular shape and with a minor presence of tails and coalescences.

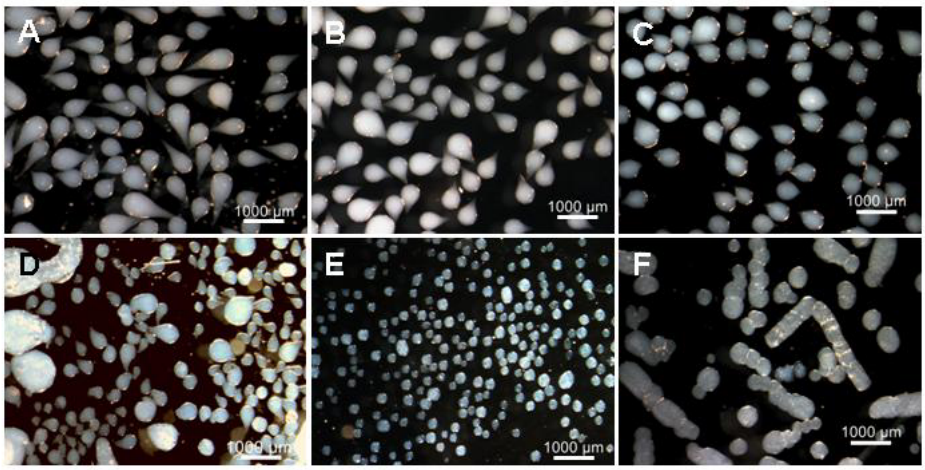


**Fig. 3. Optical microphotographs of alginate beads prepared by microfluidic squeezing approach. Microbeads prepared by external gelation procedure (A-C). The following gelling bath were employed: $BaCl_2$ solution (A); $BaCl_2$ solution plus cyclohexane (B); $BaCl_2$ solution plus cyclohexane, and glycerol in the alginate phase (C). Microbeads prepared by internal gelation procedure, effect of $BaCO_3$ concentration to the alginate solution (D-F), the following $BaCO_3$ content was employed: 5.0 mM (D); 7.5 mM (E); 10.0 mM (F).**

We also focused our attention on the preparation of alginate beads by a "partial gelation" technique. This method is based on the partial gelation of alginate by adding small amounts of $BaCl_2$ in the polymer solution, before the injection into the microfluidic device.
We investigated the effect of a different content of $BaCl_2$ on the resulting microbeads (Fig. 4 A-C). Among the different concentrations used, we obtained beads spherical in shape and homogenous in dimensional distribution only using a concentration of 0.375‰ (w/v) (Fig. 4 B).
Furthermore, the use of a microfluidic chip with a channel with a square section of 640 µm, as expected, led to an increase of particle size (Fig .4 D).

In conclusion, among the procedures available for the production of alginate microbeads, the microfluidic method appears to be one of the most appealing, allowing the production of beads with a very narrow size distribution and with different dimensions varying the microchannel size.
In this respect, the use of microfluidic procedures may open new perspectives for the encapsulation of living cells.

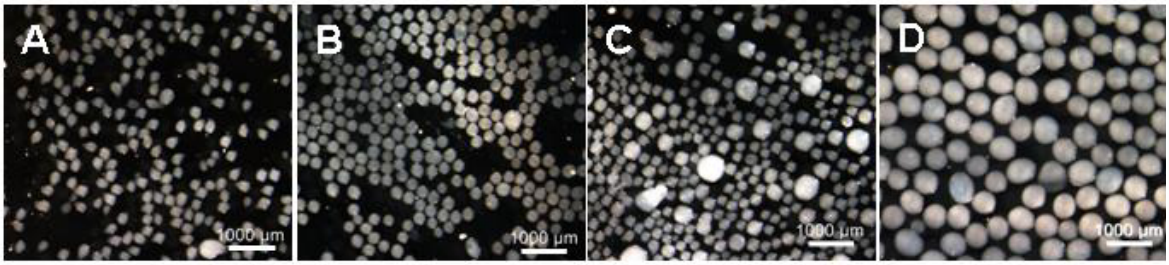


**Fig. 4. Optical microphotographs of alginate beads prepared by microfluidic squeezing approach. Microbeads prepared by partial gelation procedure. Effect of concentrations of $BaCl_2$ on the resulting microbeads (A-C): 0.3‰ (w/v) (A); 0.375‰ (w/v) (B); 0. 5‰ (w/v) (C). Microbeads prepared using a microfluidic channel with a square section of 640 µm (D).**

## References

K. Liu et al. (2006) *Shape-Controlled Production of Biodegradable Calcium Alginate Gel Microparticles Using a Novel Microfluidic Device*. Langmuir 22, 9453-9457.
N. Takasi et al. (2004) *Novel microreactors for functional polymer beads*. Chemical Engineering Journal 101, 23–29.
S. Xu et al. (2005) *Generation of monodisperse particles by using microfluidics: control over size, shape, and composition.* Angewandte Chemie-International edition, 44 (25), 724-728.
A. Martinsen et al. (1989) *Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate beads*. Biotechnol. Bioeng. 33, 79–89.
G. Coppi et al. (2002) *Protein immobilization in crosslinked alginate microparticles.* J. Microencapsul. 19, 37–44.
M. Albarghouthi et al. (2000) *Immobilization of antibodies on alginate-chitosan beads.* Int. J. Pharm. 206, 23–34.
D. Quong et al. (1998) *DNA protection from extracapsular nucleases, within chitosan- or poly-l-lysine-coated alginate beads.* Biotechnol. Bioeng. 60, 124–134.
G. Klinkenberg et al. (2001) *Cell release from alginate immobilized Lactococcus lactis ssp. lactis in chitosan and alginate coated beads.* J. Dairy Sci. 84, 1118–1127.
E. Amici et al. (2008) *Alginate gelation in microfluidic channels.* Food Hydrocolloids 22, 97–104
K-S. Huang et al. (2006) *Manipulating the genaration of Ca-alginate microspheres using microfluidic channel as a carrier of gold nanoparticles.* Lab on a chip, 6, 954-957.
D. Poncelet et al. (1992). *Production of alginate beads by emulsification internal gelation. 1. Methodology*. Applied Microbiology and Biotechnology, 38(1), 39–45.