P-084 **Novel angiogenic biotherapies for heart failure using microcapsules for the spatiotemporally-controlled delivery of growth factors**

Sébastien Banquet[1], Elodie Gomez[1], Lionel Nicol[1], Florence Edwards-Lévy #[2], Jean-Paul Henry[1], Damien Schapman[3], Brigitte Dautreaux[1], Françoise Lallemand[1], Fabrice Bauer[1], Vincent Richard[1], Paul Mulder[1], Christian Thuillez[1], Ebba Brakenhielm[1]
[1]INSERM U644, Rouen University Medical School, France, [2]CNRS UMR 6229, Faculty of Pharmacy, Reims University, France, [3]Platform for Cell Imaging, European Institute for Peptide Research , Rouen University, France.
Florence.edwards@univ-reims.fr

INTRODUCTION AND OBJECTIVES

Therapeutic angiogenesis is a promising approach for the treatment of cardiovascular diseases, including myocardial infarction (MI) and chronic heart failure. The main problems of current proangiogenic approaches include the limited duration of the therapy achieved with bolus delivery of naked proteins, the high costs associated with protein therapy because of the need for large doses, the transient effects of treatment likely due to the generation of unstable blood vessels, and important safety issues related to the possibility of inadvertent stimulation of angiogenesis in distant, dormant micrometastases.
In this study we aimed to improve proangiogenic therapies by developing microcapsules as injectable delivery systems for spatiotemporally controlled release of a growth factor combination, and evaluating functional consequences of targeted intramyocardial growth factor delivery in chronic heart failure (Banquet 2011).

MATERIALS AND METHODS

Albumin-alginate microcapsules

Albumin-alginate microcapsules were prepared using a modified version of the previously described interfacial cross-linking method (Lévy 1991). Briefly, 4% human serum albumin and 2% propylene glycol alginate were dissolved in a phosphate buffer pH 7.4. This aqueous phase was emulsified in cyclohexane containing 2% sorbitan trioleate, at a stirring speed of 2000 rpm. Then, a 2.5% solution of terephthaloyl chloride in a chloroform-cyclohexane mixture was added to the emulsion and the cross-linking reaction was allowed to develop for 30 min. The microcapsules were separated from the organic phase by centrifugation, and washed.Diameter measurements were performed using laser diffraction (Particle Sizer LS200, Beckman-Coulter). After staining with methylene blue, the microparticles were observed with a light microscope (Olympus, BH-2) equipped with interferential phase contrast. SEM observations (JSM-5400LV, JEOL) were made after alcohol dehydration of microcapsule suspension followed by Au/Pd coating. Finally, the microcapsules were freeze-dried in a Freezone 6 (Lab-Conco).

In vitro growth factor release

Briefly, lyophilized microcapsules were loaded with growth factors by imbibition (Hurteaux 2005), with 1 µg growth factor per 1 mg microcapsules. Growth factor release after incubation in extracellular fluid mimetic release buffer was quantified by ELISA. Data are presented as mean amount (nanograms) of growth factor released per day per 1 mg microcapsules (n=3).

Matrigel Plug Model

Briefly, 500 µL Matrigel was mixed with microcapsules loaded with growth factors as above. Controls contained Matrigel mixed with buffer or microcapsules without growth factors. The Matrigel mixture was subcutaneously injected in male Balb/c mice (n=5 per group). After 3 weeks, Matrigel plugs were harvested and snap-frozen.

Chronic Heart Failure Model

We induced MI in Wistar rats by ligation of the proximal left coronary artery after a left thoracotomy (n=22 rats per group)(Mulder 2004). Immediately after ligation, microcapsules loaded with growth factors were injected in 3 spots (23 µL per spot) along the infarct border zone. The total amount of growth factor administered per heart was 125 ng HGF or 500 ng FGF-2 alone or in combination. Controls were injected with the same numbers of microcapsules without growth factor. At the time of death, hearts were arrested in diastole by immersion in ice-cold saturated potassium chloride buffer.

Confocal Imaging of Growth Factor Distribution

Recombinant human FGF-2 or recombinant mouse HGF (20 µg) was fluorescently labeled with an Alexa-555. Lyophilized microcapsules were loaded with fluorescent growth factors as above. Microcapsules were either imaged directly by confocal microscopy or injected after coronary ligation in rats as above. Cardiac samples retrieved at 1 or 3 weeks after MI were imaged with a Leica SP5 TCS X inverted confocal microscope.

Magnetic Resonance Imaging

Cardiac perfusion was assessed by arterial spin-labeling magnetic resonance imaging with a 4.7-T small animal magnet (Biospec 47/40 advanced II, Brucker, Ettlingen, Germany). Briefly, the perfusion sequence was run in the short-axis plane, allowing determination of myocardial tissue perfusion. Global and slice-selective spin inversion recovery T1* (fitted time constant) maps were acquired. Perfusion images were analyzed with ParaVision 5.0 software (Brucker) by 2 independent observers, and regional perfusion in the treated area of the LV was calculated as described.

Echocardiography

Animals (n=14 to 15 rats per group and 8 age-matched shamoperated rats) were examined at 1 and 3 months after MI by transthoracic echocardiography as previously described (Mulder 2004). Measurements performed by a single echocardiographer blinded to the treatment groups were made in accordance with the conventions of the American Society of Echocardiography.

RESULTS AND DISCUSSION

Alginate is suitable for the delivery of positively charged proteins such as FGF-2 and HGF because it bears negatively charged carboxylic groups available for electrostatic interactions. Covalently cross-linking polysaccharides and proteins in a microcapsule membrane prevents hydrolysis-driven dissolution and delays protease-driven degradation, resulting in more stable particles with reproducible drug release rates. Thus, we developed microcapsules containing a thin, covalently cross-linked human serum albumin and propylene glycol alginate membrane surrounding a liquid center. Laser diffraction measurements and microscopic observations revealed that these albumin-alginate microcapsules had a mean diameter of 100 µm and were roughly spherical. After dehydration, they presented a pleated surface as observed by scanning electron microscopy (SEM, Figure 1).

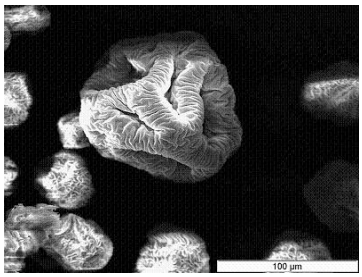

Figure 1: SEM of the dehydrated microcapsules.

The microcapsules were assayed *in vitro* for the release of angiogenic growth factors under conditions approximating the *in vivo* tissue environment. Whereas FGF-2 release from the microcapsules began immediately, the release of HGF was delayed for 1 week (Figure 2). The whole release period lasted 6 weeks.

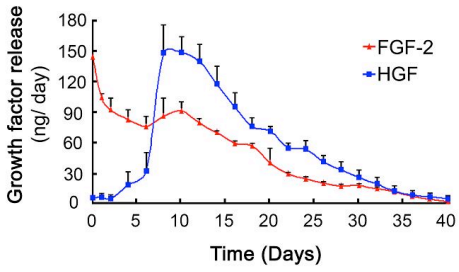

Figure 2: Release kinetics of FGF-2 and HGF from the microcapsules.

Confocal analyses of microcapsules loaded with fluorescently labelled growth factors show that both growth factors bound to the microcapsule surface layer, confirming their interactions with the cross-linked protein-polysaccharide membrane. FGF-2 was also found in the liquid center of the microcapsule. These findings may in part explain why the microcapsules display different release profiles for FGF-2 and HGF.

Next, we compared treatment with growth factor–loaded microcapsules and naked growth factors using the mouse Matrigel plug model. Growth factor delivery by microcapsules was found to be 3 to 6 times more potent to induce angiogenesis compared with bolus delivery of growth factors (Figure 3).

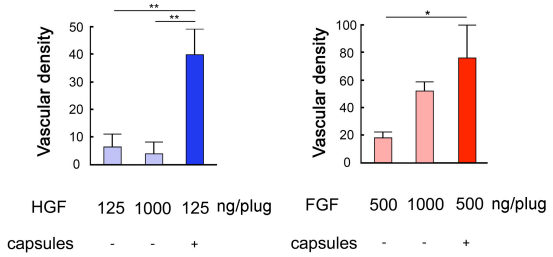

Figure 3: Vascular density in Matrigel plugs as a function of growth factor dose and delivery system.

Finally, in a rat model of chronic heart failure induced by coronary ligation (n=14 to 15 rats per group), we found that intramyocardial slow release of fibroblast growth factor-2 with hepatocyte growth factor potently stimulates angiogenesis and arteriogenesis, leading to improved cardiac perfusion after 3 months, as shown by magnetic resonance imaging. These multiple beneficial effects resulted in reduced adverse cardiac remodeling and improved left ventricular function, as revealed by echocardiography.

CONCLUSIONS

The albumin-alginate-based injectable microcapsules developed in this study seem well adapted for intramyocardial delivery of angiogenic growth factors for therapeutic angiogenic approaches to ameliorate cardiac function following myocardial infarction.

REFERENCES

- Banquet S. et al. (2011) *Arteriogenic therapy by intramyocardial sustained delivery of a novel growth factor combination prevents chronic heart failure*. Circulation 124: 1059-1069.
- Lévy MC et al. (1991) *Mixed-wall microcapsules made of cross-linked proteins and polysaccharides: preparation and properties*. J Microencapsul. 8(3) 335-347.
- Hurteaux R et al. (2005) *Coating alginate microspheres with a serum albumin-alginate membrane: application to the encapsulation of a peptide*. Eur J Pharm Sci. 24(2-3)187-197.
- Mulder P et al. (2004) *Long-term heart rate reduction induced by the selective I(f) current inhibitor ivabradine improves left ventricular function and intrinsic myocardial structure in congestive heart failure*. Circulation 109:1674 –1679.