

POLYSACCHARIDE-BASED HYDROGEL FOR SMOOTH MUSCLE CELL CULTURE

[A. Autissier](#)^{1,2}, [D. Letourneur](#)², [C. Le Visage](#)²

¹ Faculté de Chirurgie-Dentaire Université Paris 5, ² [Inserm, U698, Bio-Ingénierie](#), CHU Xavier Bichat, Bât. Inserm, 46 Rue Henri Huchard, F-75877 Paris Cedex 18, France

INTRODUCTION: Cardiovascular diseases related to vessel wall thickening may require surgery techniques such as multiple bypasses. Unfortunately, healthy vascular tissue from the patient is not always available to carry out this type of graft. Recent investigations in the tissue engineering field have aimed at finding biocompatible materials and scaffolds that could be seeded with cells and used as vascular substitutes¹. To this end, we developed biodegradable polysaccharide-based hydrogels² that could serve as scaffolds for vascular engineering.

METHODS: Polysaccharide-based hydrogels were prepared using pullulan (Mw 200,000, Hayashibara Inc.). Chemical crosslinking was carried out using the reticulating agent STMP (trisodium trimetaphosphate, Sigma). The mixture was poured into Petri dishes and incubated at 50°C for 20 min. The resulting gels were washed in PBS pH 7.4 and a circular punch was used to cut 6 mm diameter-scaffolds. The internal structure of the gels was analyzed using ESEM (Environmental Scanning Electron Microscope). Rabbit Smooth Muscle Cells (SMC Rb1 line) were seeded on the top of the gels (10^5 cell/cm²) then maintained in culture for up to 7 days in DMEM with 10% Fetal Bovine Serum. In some experiments, cells were labeled prior to the seeding step with a fluorescent dye (PKH26, Sigma). Cellular adhesion to the gels was evaluated by direct observation by light microscopy, fluorescence microscopy and confocal microscopy. Cell viability at day 4 was assessed using a Live/Dead assay (Calbiochem). A metabolic assay (MTT) was performed at days 1, 4 and 7 to determine the total number of cells per gel. Gel samples were fixed in formaldehyde, OCT-embedded then cryosectioned. Histological stainings were carried out to localize the cells within the gels.

RESULTS: Pullulan solution was cross-linked in order to obtain thin discs designed for cell culture support. This polysaccharide gave homogeneous, transparent and easy to handle gels (Fig.1A). Water content was found to be higher than 90%. Environmental SEM analysis revealed a smooth surface, on which SMCs were successfully seeded. Fall-out cells were observed but, overall, more

than 50% of the initial cells remained associated to the gels. Cells attached in less than 2 hours. Cells were observed spreading out on the gel surface, forming numerous links between the initial cell aggregates (Fig.1B). The absence of toxicity of the gels was evidenced by the high viability of the cells. Histological observations confirmed the presence of cells in the scaffolds.

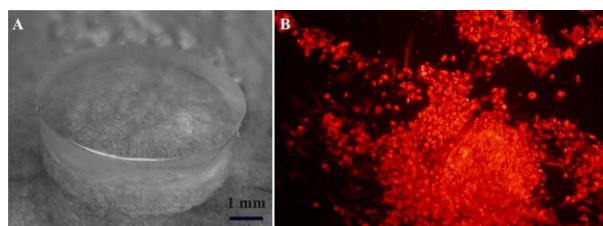


Fig. 1: (A) Transparent and easy to handle gel (disc, 6 mm diameter). (B) Fluorescent labeling of the SMCs prior to their seeding enabled their morphology and distribution to be observed.

A metabolic assay (MTT) demonstrated the cell proliferation over time with a progressive increase of the cell density from day 1 to day 7 (Fig.2).

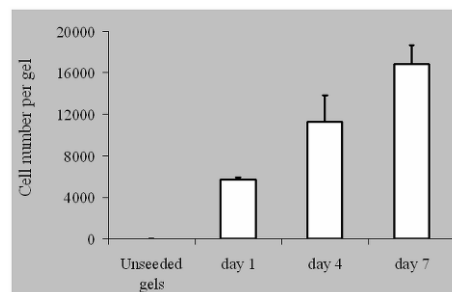


Fig. 2: MTT assay results expressed as the mean value of the total cell number per gel \pm SEM (n=4)

DISCUSSION & CONCLUSIONS: We prepared biodegradable polysaccharide-based hydrogels that could serve as scaffolds for vascular engineering. Hydrogels allowed SMC adhesion, spreading and proliferation. Future studies will focus on the culture of vascular cells on tubular-shaped hydrogels and *in vivo* implantation.

REFERENCES: ¹J.M. Chupa *et al.* (2000) *Biomaterials* **21**:2315-22. ²D. Letourneur *et al.* (2002) *JBMR* **60**:94-100.