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PERCOLATING HYDROGELS FOR TISSUE ENGINEERING

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INTRODUCTION: Chitosan is a linear copolymer of linked β(1→4) glucosamine and N-acetyl glucosamine. Although present in biomass, it is produced from chitin, a glycosaminoglycan, extensively widespread on earth. Indeed, the latter constitutes the structure polymer of the cuticles of all arthropods and endoskeletons of all cephalopods, etc. It is noteworthy to observe that both chitin and chitosan are completely absent in mammals.

Numerous results reported in the literature reveal that chitosan exhibits very interesting biological properties, which can be summarized by the fact that this polysaccharide is a bioactive biopolymer useful for tissue regeneration[1]. In the field of cartilage some results on chitosan films have demonstrated its capability to favor the chondrocyte proliferation, but with an elicitation of the production of both collagens 1 and 2[2].

Our work consists in the introduction of a new concept of biomaterials for tissue engineering, based on the processing of chitosan physical hydrogels with a well defined and appropriate chemical structure and morphology. These materials constitute "decoys" for biological media. Our work also consists to show that this concept completely disagrees with the old theory of the "scaffold". Thus, the formation of an appropriate interface between the surface of the material and the living media is sufficient to allow the induction of a tidy neo-tissue.

METHODS: All the materials were produced from a unique batch of chitosan obtained from squid pens, kindly provided by France Chitine. Its degree of acetylation (DA) was 5.2% and the weight-average molecular weight close to 420.000 g/mol. In order to study the role of DA, the sample was reacetylated as described previously[3]. Then, we disposed of a series of polymers of same molecular dimensions but with DA's varying within 5.2-60%.

Gelation of chitosan was made from solutions in an hydroalcoholic media subjected to an evaporation at 40°C up to observe the gelation. The gels were then transferred into an alkaline solution to convert the ammonium groups of glucosamine residues into free amines. After a thorough washing in distilled water, a true physical hydrogel only constituted of a hydrated polymer network at less than 5% was obtained. This gel was then sterilized by wet heating at 120°C for 20 min. The morphology of the gels was studied both by atomic force- and scanning electron-microscopies.

These gels were then subjected to a cell culture media and thus were ready for experiments of chondrocyte cultivation (see M. Corvol et Al.).

RESULTS AND DISCUSSION: Gelation of chitosan solutions is observed when two conditions are verified. 1- the polymer concentration must be initially over the critical concentration of chain entanglement C*; 2- a critical value of the balance between attractive and repulsive interactions must be achieved. This situation occurs when a percolating condition of gelation takes place in the media. Two examples leading to this situation have been produced in our laboratory. The role of various parameters related to the chemical structure, especially the DA of the polymer and/or to its environment were studied.

The gel morphology has been observed both by atomic force- and scanning electron- microscopies. We show that as in the case of most living tissues, chitosan physical gels are three-dimensional networks quite different from sponge-like systems. The dimension of the pores whether on the surface or in the bulk of the material preclude any penetration by living cells.

These gels can be easily sterilized by wet drying at 120°C. This treatment, contrary to γ irradiation, has no significant influence on the properties of the material, especially as concerns the preservation of the chemical structure, molecular weight and

morphology of the gel.

In order to favour the interaction between this gel and living media, in particular with the surrounding material of chondrocytes, we also prepared microgels thus increasing the surface potentially in contact with this material. Chitosan microgels were then prepared by crushing of macrogels before to be subjected to chondrocyte cell cultures (See M. Corvol et Al.). This kind of material bears various characteristics allowing a behaviour of decoy of living media by its structure and morphology.

CONCLUSION: It is possible to process micro-hydrogels of chitosan which surface in contact with living cells constitutes a decoy on which an appropriate bioinductive interface can be generated. This interface, by the nature of both the structures and the kind of interaction involved in its formation generates favourable responses from the cells leading to a continuous remodelling of the interface allowing then the production and deposition of an extracellular matrix and/or cell proliferation.

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