



## **Production of gellan gum scaffolds via cheap and sustainable fabrication methods**

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### **Introduction**

Gellan gum is a bacterial polysaccharide, obtained from *Sphingomonas elodea*, which is composed by a tetrasaccharide repeating sequence of two residues of  $\beta$ -D-Glucose, one of  $\beta$ -D-Glucuronate and one of  $\alpha$ -L-Rhamnose. In our work, we developed a sustainable procedure for the preparation of biodegradable and mechanically modifiable scaffolds for tissue engineering. In the fabrication route, gellan gum is combined with different saline solutions that act as crosslinkers leading to the formation of a sponge, which is ultimately obtained via lyophilization. Our methodology leverages the ionic crosslinking of low-acyl gellan gum to obtain a flexible system for cell culture produced with a low impact approach.

### **Experimental**

Gellan gum (Phytigel, Sigma Aldrich) was dissolved in water at 90°C in a concentration of 1.25 w/v% and left under stirring for 4 h at 400 rpm. The solutions were then autoclaved and mixed at 90°C with Phosphate Buffer Saline (PBS 1×) without Ca<sup>2+</sup> and Mg<sup>2+</sup> in a volume ratio 1.00/0.16 and cooled down to room temperature at different cooling rates. Thereafter, the gel was incubated in a PBS 1× solution to saturate the uncrosslinked acidic group for 30 h-48 h. The last steps consisted in freezing for 72 h at -80°C and then freeze-drying until a dry product was obtained. A morphological analysis was performed via scanning electron microscopy (SEM) and a mechanical analysis was carried out via dynamic-mechanic-thermal analysis (DMTA) at T = 20°C.



### **Results and Discussion**

Micrographs obtained by SEM showed a network of interconnected pores with mean pore diameter of  $264 \pm 103 \mu\text{m}$ , with a distribution ranging in  $150 - 474 \mu\text{m}$ . Mechanical properties were also investigated through DMTA, showing very soft materials with a storage modulus at a frequency of 1 Hz of  $37 \pm 9 \text{ kPa}$ . Scaffold stability was tested by keeping the scaffold at  $37^\circ\text{C}$  for 7 d in alpha-minimum essential medium ( $\alpha$ -MEM), while its dissolution was achieved by cation chelation, via either incubation in ethylenediaminetetraacetic acid disodium salt (EDTA) aqueous solution at 0.25% w/v, or thermally in PBS 1 $\times$  at  $T > 45^\circ \text{C}$ .

### **Conclusions**

Gellan gum sponges were fabricated in water solutions followed by freeze-drying, thus using a low environmental impact approach which make them suitable for subsequent applications in soft tissue engineering, also corroborated by their stability in simulated cell culture conditions. In addition, an efficient dissolution of the scaffolds can be achieved via cation chelation, which could be useful to extract the cells after their culture. However, milder dissolution conditions should be investigated to prevent cellular damages.

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### **Biography**

Emanuele Bosurgi obtained his bachelor in Industrial Chemistry (Sapienza, University of Rome) and is currently pursuing his thesis as a student of the master "Materials and Nanotechnology" at the Department of Civil and Industrial Engineering (University of Pisa).

His scientific interests extend from the development and characterization of hydrogel, microparticles and scaffolds for tissue engineering, and imaging of biological samples with confocal laser scanning microscopy.