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Chitosan-Alginate Polyelectrolyte Complex Scaffolds by Additive Manufacturing for 3D *In Vitro* Modelling of Ovarian Cancer

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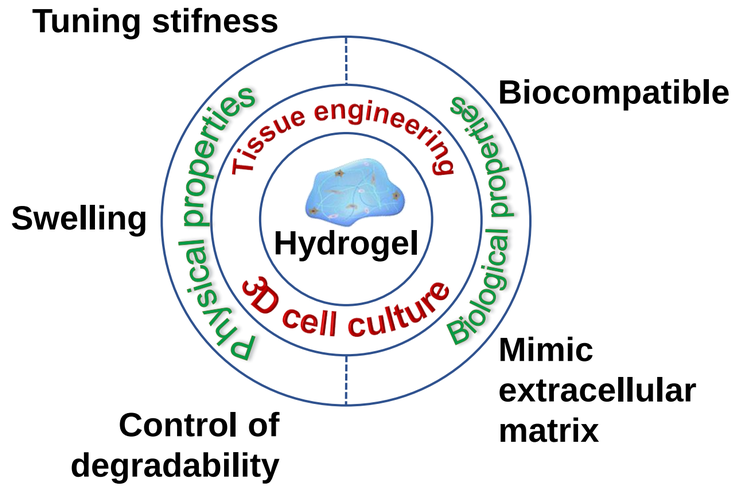
BACKGROUND

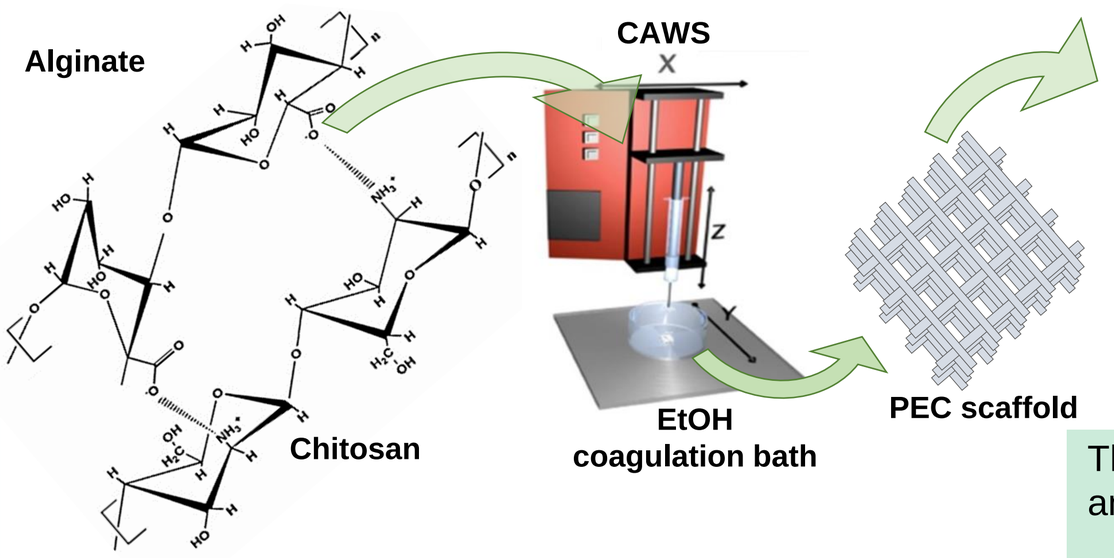
Hydrogels have been used over the past two decades as one of the most common types of tissue engineering scaffold thanks to their ability to maintain a distinct 3D structure in physiological environment as support for cells in engineered tissues. The high-water content absorbed by hydrogels can provide an ideal environment for cell survival, mimicking that in many native tissues [1]. One of the most common methods for hydrogel preparation is through physical crosslinking of hydrophilic macromolecular chains. **Polyelectrolyte complexes (PECs)** are formed by electrostatic interactions between oppositely charged groups present along the backbones of two polyions. PECs between polymers from natural sources have been widely investigated in tissue engineering to exploit their biocompatibility, controlled biodegradability, and tuneable mechanical properties [2].

In the present study, a novel **Additive Manufacturing (AM)** approach for the preparation of porous hydrogels made of a **PEC between chitosan (Cs) and alginate (Alg)** was investigated. The developed PEC hydrogels are currently investigated as scaffolds for ***in vitro* 3D modelling of human ovarian cancer**.

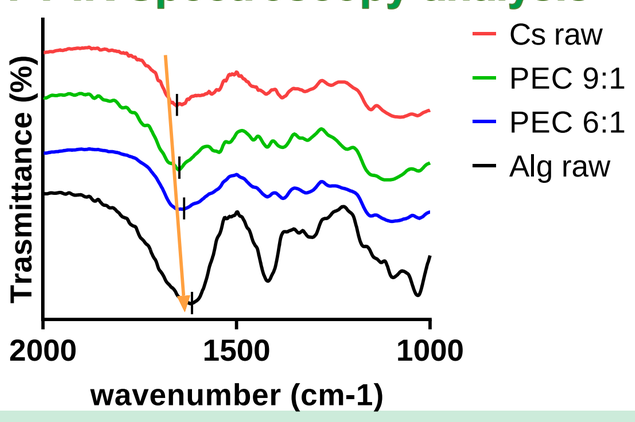


PEC HYDROGELS FABRICATION AND CHARACTERIZATION

Computer-Aided Wet Spinning (**CAWS**) is an AM technique allowing the fabrication of scaffolds with advanced control over external geometry and porous structure through the extrusion of a polymeric solution/suspension in a coagulation bath [3]. Controlled extrusion of Cs/Alg aqueous suspensions into ethanol was demonstrated to be a suitable approach for the fabrication of PEC hydrogels with a macroporous architecture.

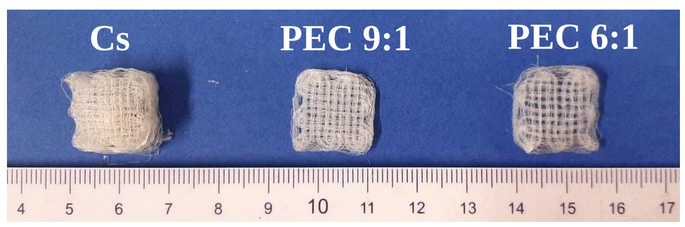


FT-IR Spectroscopy analysis

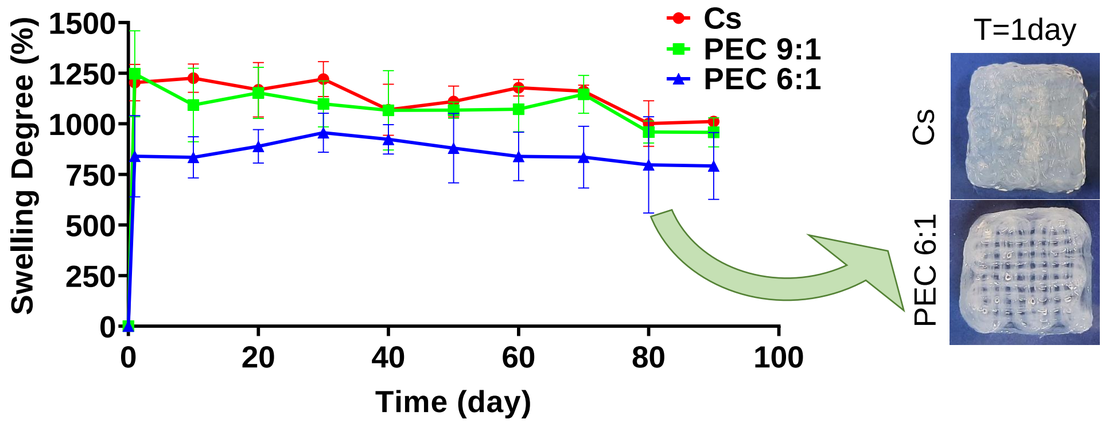


The interaction between Cs amino groups and Alg carboxyl groups leads to shift and intensification of Cs amide peaks.

CAWS parameters were investigated and optimized for the fabrication of scaffolds based on i) Cs ionically crosslinked with sodium tripolyphosphate (TPP) or (ii) PECs with different Cs/Alg ratios (9:1 or 6:1 wt.).

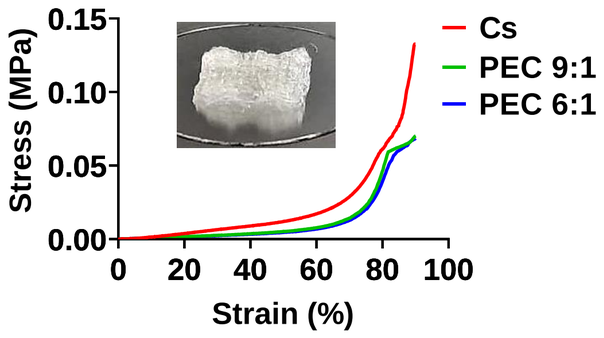


Scaffolds stability in cell culture medium



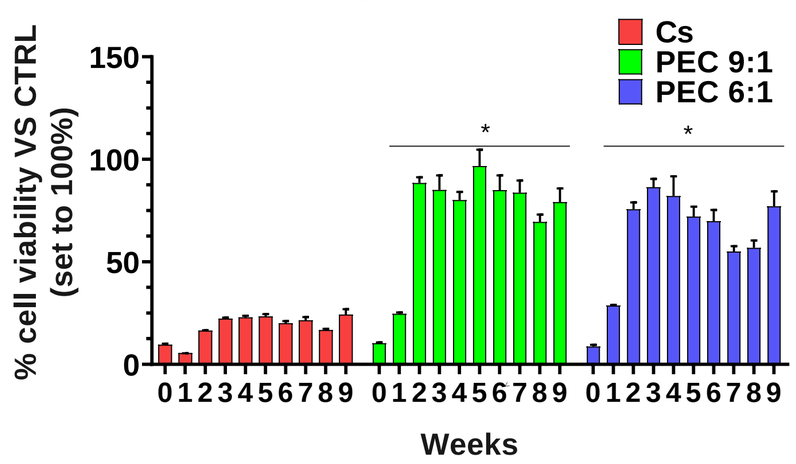
Swelling degree increases as the degree of complex formation decreases. Hydrogels were **stable in cell culture medium** at 37 °C for a period of 90 days.

Compressive mechanical properties

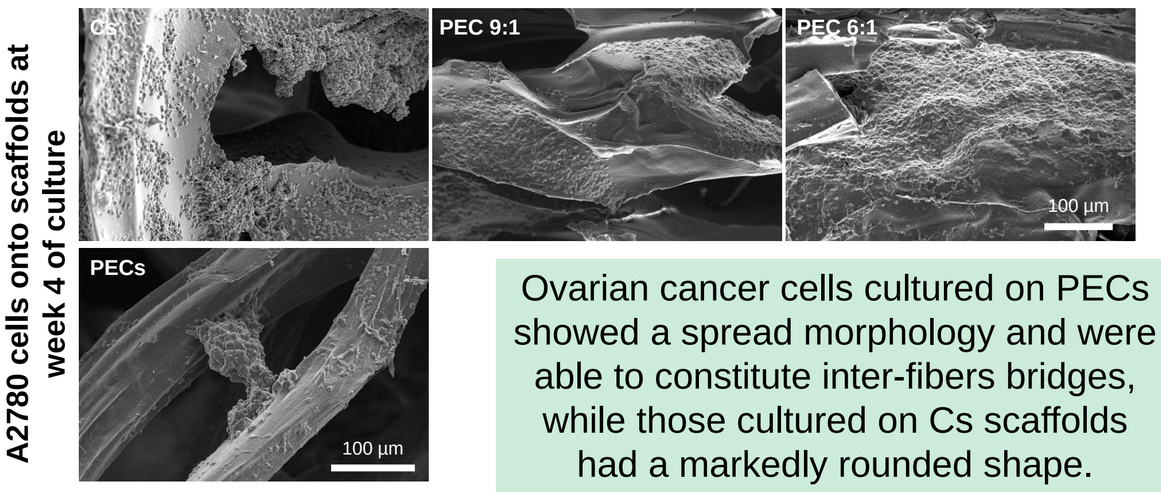


Cs samples were stiffer than PECs ones, as a consequence of the different fabrication parameters employed.

Biological investigations



All hydrogels support the **A2780 human ovarian cancer cell line** proliferation for 9 weeks; PEC samples showed a significantly higher cell viability.



Ovarian cancer cells cultured on PECs showed a spread morphology and were able to constitute inter-fibers bridges, while those cultured on Cs scaffolds had a markedly rounded shape.

CONCLUSIONS

- An innovative approach to the preparation of Cs/Alg PEC scaffolds suitable for *in vitro* cell growth was developed and exploited for 3D ovarian cancer modelling.
- PEC scaffolds characterization highlighted electrostatic interactions between oppositely charged groups of the two polyions, as well as a sample structural stability in cell culture medium suitable for long-term 3D *in vitro* biological investigations.
- Biological characterization carried out with the A2780 ovarian cancer cell line highlighted interesting differences in cell adhesion and colonization among investigated hydrogels with different composition, that could be correlated to the different scaffold's mechanical properties.

REFERENCES

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[3] D. Puppi, et al., *Polym. Int*., 66, 1690-1696 (2017).