Hydrogel scaffolds based on k-Carrageenan/xyloglucan blends to host spheroids from human adipose stem cells

M.A. Sabatino[1], A.B. Di Stefano[2], E. Muscolino[1], F. Grisafi[2], L.A. Ditta[3], D. Giacomazza[3], A. Cordova[2], F. Moschella[2], C. Dispenza[1]

[1]Dipartimento di Ingegneria, Università degli Studi di Palermo, Viale delle Scienze 6, 90128 Palermo, Italy.
[2]Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche, Università degli Studi di Palermo, via del Vespro 129, 90127, Palermo, Italy
[3] Istituto di BioFisica, CNR, Via U. La Malfa 153, 90146 Palermo, Italy.

emanuela.muscolino@unipa.it

INTRODUCTION

Hydrogels are water-swollen networks of hydrophilic polymer. They can be fabricated in various shapes and swell in water or aqueous solutions maintaining their original shape or undergo progressive erosion; can exibit large volume phase transitions with the change of one environmental parameter (stimuli-responsivness), shock absorption and low sliding friction properties (1). The morphology and mechanical properties of hydrogels are strongly affected by the network composition, the nature and degree of crosslinking and the degree of swelling. Indeed, when hydrogels are designed as scaffolds for human tissues remodeling, they must have sufficient mechanical integrity to provide support to the cells from the time of implantation to the completion of the process. The large amount of water present in the hydrogels and its microscopic pores interconnectivity allows transportation of nutrients, oxygen and metabolites, that ensures cells viability, and permits cells migration and scaffold colonization. The polymeric network can immobilize biomolecules that may affect cells growth or differentiation, control drug release profiles and enzymatic degradation (2,3). The combination of two hydrogel-forming polymers with different chemistries and crosslinking densities can be used to tailor the morphology, mechanical strength and toughness of the scaffold to meet specific requirements (1). This work investigates the physico-chemical, morphological and mechanical properties of hydrogels formed by the blend of two polysaccharides, k-Carrageenan (k-C) and Degalactosylated Xyloglucan (Deg-XG) undergoing salt-induced and temperature-induced sol-gel transition, respectively. It also studies the compatibility of the two biopolymers with spheroids from adipose-derived stem cells (S-ASCs) in the prospect of developing instructive scaffolds for use in regenerative medicine.

EXPERIMENTAL

k-C was provided by Gelcarin ME 8625 FMC Biopolymer; Deg-XG was provided by DSP Gokyo (Japan), KCl were purchased from Sigma Aldrich; S-ASCs were obtained from liposuction of healthy patients.

Hydrogels of k-C(2, 3%w), Deg-XG(3%w) and their blends obtained keeping constant the concentration of k-C (2%w) and varing the concentration of Deg-XG (0.2, 0.5, 0.75, 1%w) were produced. KCl (0.4%w) was used to crosslink k-C.

Swelling and erosion measurments in buffer phosphate (PBS) were performed by immerging hydrogel in the solution and weighting the samples at given intervals.

Frequency-sweep test was carried out using a stress-controlled Rheometer AR G2 (TA Instruments), at 37°C, in the frequency range 0.5-50 Hz and with a strain at 8×10^{-4}.

Morphological investigations were carried out using Field Emission Scanning Electron Microscope (FESEM-JEOL) at 10kV on freeze-dried hydrogel samples.

Preliminary compatibility test with S-ASCs were performed by mixing the cells and their culture medium with Deg-XG 3%w and k-C 2% and morphologically evalueting the viability by using the optical microscope after 21 days.

RESULTS AND DISCUSSION

Swelling/erosion test performed in PBS buffer at 37°C on all k-C containing systems show almost no further swelling (max. 1%) followed by slow erosion (-10% in 5 weeks). The swelling degree for the blends is not significantly affected by the Deg-XG content.

Fig. 1a reports the storage modulus (G') and the loss modulus (G") as function of the frequency for k-C (2% and 3%), Deg-XG 3% and k-C 2%/Deg-XG 1% hydrogels. For all k-C containing systems, G' is more than one order of magnitude higher than G" and invariant with frequency. This is the typical behaviour of fairly strong gels. G' is higher than G" also for Deg-XG 3%, but G" is frequency dependent and G' is almost two order of magnitude lower than for the k-C system at the same polymer concentration. The k-C 2%/Deg-XG 1% blend shows intermediate values of G' and G".

SEM analysis shows for k-C 2% (Fig. 1b) stacks of corrugated lamellae, for Deg-XG 3% (Fig. 1c) a very heterogenous structure, with large cavities and small pores. The k-C 2%/Deg-XG 1% (Fig. 1d) shows a fairly uniform, open and interconnected microporous structure. S-ASCs have been incubated with k-C and Deg-XG hydrogels. In both cases the stem cells spheroids retain their pristine morphological structure.

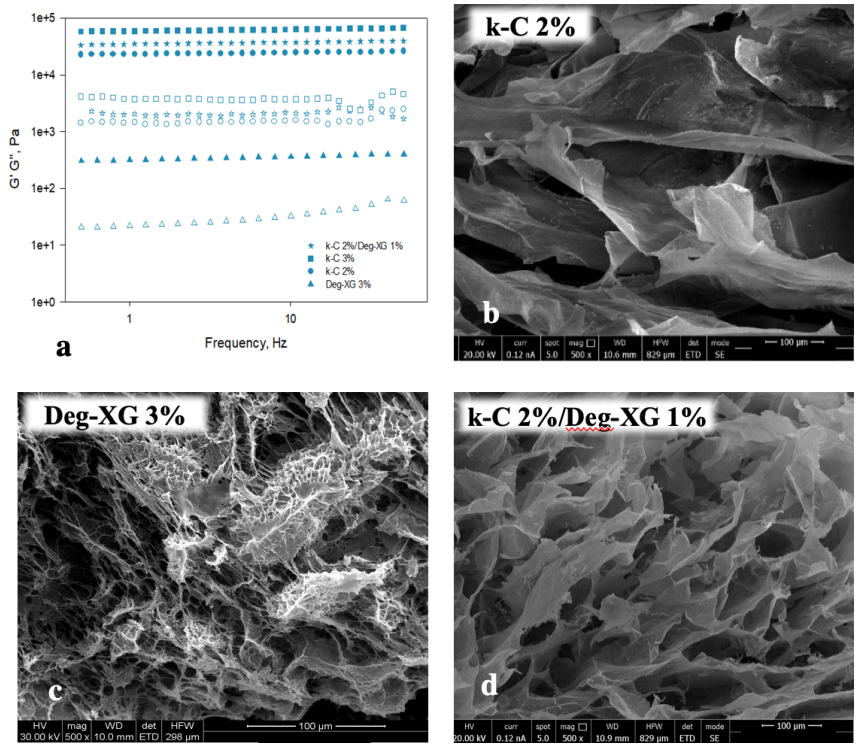

Fig. 1: a) Frequency-sweep analysis (G' full dot, G" hollow dot) on the systems with only k-C (2% and 3%) and the k-C 2%/Deg-XG 1% polymer blend; b) SEM images of k-C 2% hydrogel; c) SEM images of k-C 2%/Deg-XG 1% hydrogel; d) SEM images of Deg-XG 3% hydrogel.

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