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Biomimetic glycerohydrogel materials based on chitosan L- and D-aspartate

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INTRODUCTION & AIM

Biomimetic sol–gel synthesis using alkoxysilane derivatives (gel precursors) and natural polysaccharides (templates) is one of the current directions for obtaining hybrid hydrogel materials for medical purposes.

The aim of this work is to study the surface morphology, the level of supramolecular structuring of glycerohydrogel plates based on CS·L-(D-)AspA obtained by biomimetic sol-gel synthesis and to evaluate their biological activity.

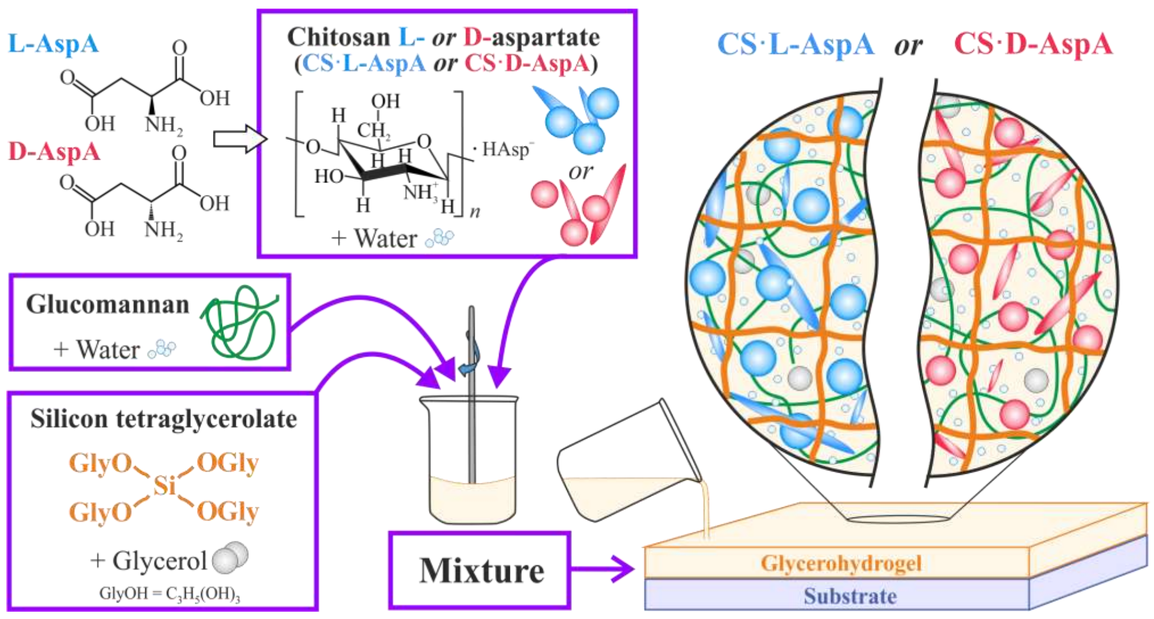

Fig. 1. Scheme of preparing our glycerohydrogel plates based on CS·L-AspA and CS·D-AspA

METHOD

In this work, polymeric glycerohydrogels in the form of thin-film plates were obtained by biomimetic sol–gel synthesis using pharmacologically active silicon tetraglycerolate, biologically active chitosan L-(D-)aspartate (CS·L-(D-)AspA), and biotolerant glucomannan. The surface microrelief of the samples was examined by atomic force microscopy, and the level of supramolecular structuring of their polymer phase was assessed by X-ray diffractometry. A comparative analysis of the adhesion, spreading and proliferation rate *in vitro* of epithelial-like cells of the rhesus macaque embryonic kidney MA-104 and epithelial cells of human fibroblasts and keratinocytes in the presence of CS·L-(D-)AspA was carried out.

The addition of CS·L-(D-)AspA to the nutrient medium to cultivate MA-104 epithelial cells, human fibroblasts and keratinocytes significantly accelerates the adhesive and proliferative activity *in vitro* of the cell cultures tested.

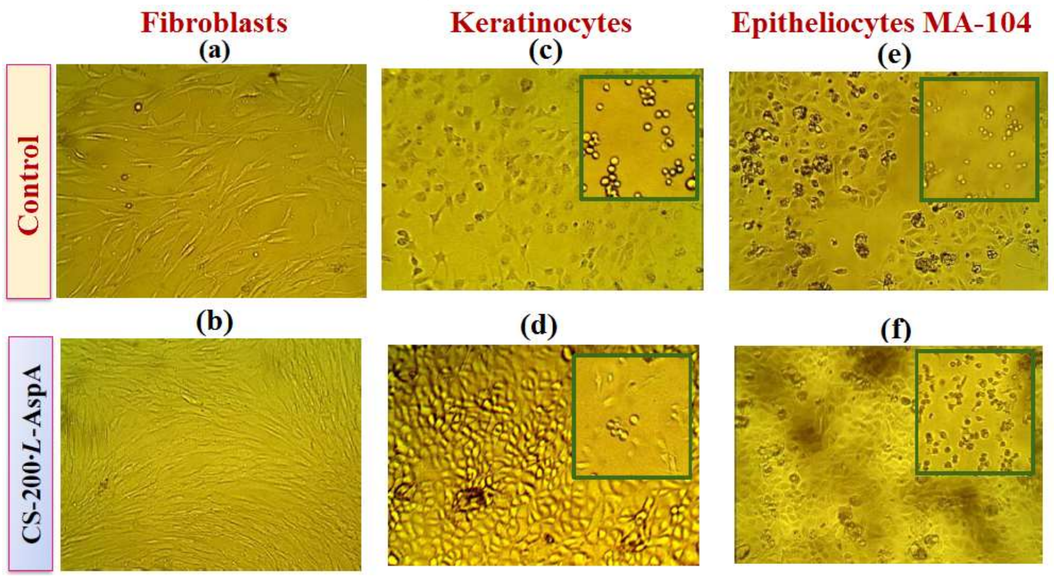

Fig. 3. Formation of a monolayer of dermal fibroblasts (a, b), keratinocytes (c, d) and epithelial cells of the MA-104 line (e, f) after an hour (insets) and 48 hours of cultivation in a nutrient medium without (a, c, e) and with the addition of CS-200·L-AspA; $C_{CS\text{-}200}$ = 0.05 g/dl, dilution 1:6, [AspA]/[~-NH_2] = 0.8 mol/mol of NH_2.

Table 2. Comparison of the nature of the proliferative effect in vitro on the cell lines of epithelial-like cells MA-104 and epithelial cells of human fibroblasts and keratinocytes, t = 24 hours, C_{CS} = 0.05 g/dl, dilution 1:6, [AspA]/[~-NH_2] = 0.8 mol/mol of NH_2 CS•L-AspA.

Cells	[–NH_2]:[Acid], mol of NH_2 : mol	Number of adherent cells, %	Number of spread cells, %	Monolayer formation time, hour	Proliferation index
		t = 1 hour			t = 48 hour
Keratinocytes	1.0:0.8	51.4	91.5	<56	3.1
Fibroblasts		92.4	96.5	<48	2.7
Epitheliocytes MA-104		99.3	97.7		4.5

RESULTS & DISCUSSION

It has been established that our glycerohydrogel plates based on CS·L-AspA and CS·D-AspA are represented by interpenetrating spatial networks of both organic and inorganic nature, filled with a water–glycerol medium.

The samples are characterized by a complex surface topography with two types of structural irregularities. For the CS·L-AspA plates, a predominantly “needle-like” relief is visualized with a predominance of protrusions up to 4.2 μm high, while a “needle-grained” relief is characteristic for the CS·D-AspA ones with protrusions up to 2.8 μm high and pores with diameters of ~3–10 μm. The CS·L-AspA plate sample had the most developed surface with the largest protrusion height and pore depth. The surface of our CS·D-AspA sample was significantly more asymmetrical and had a higher kurtosis value.

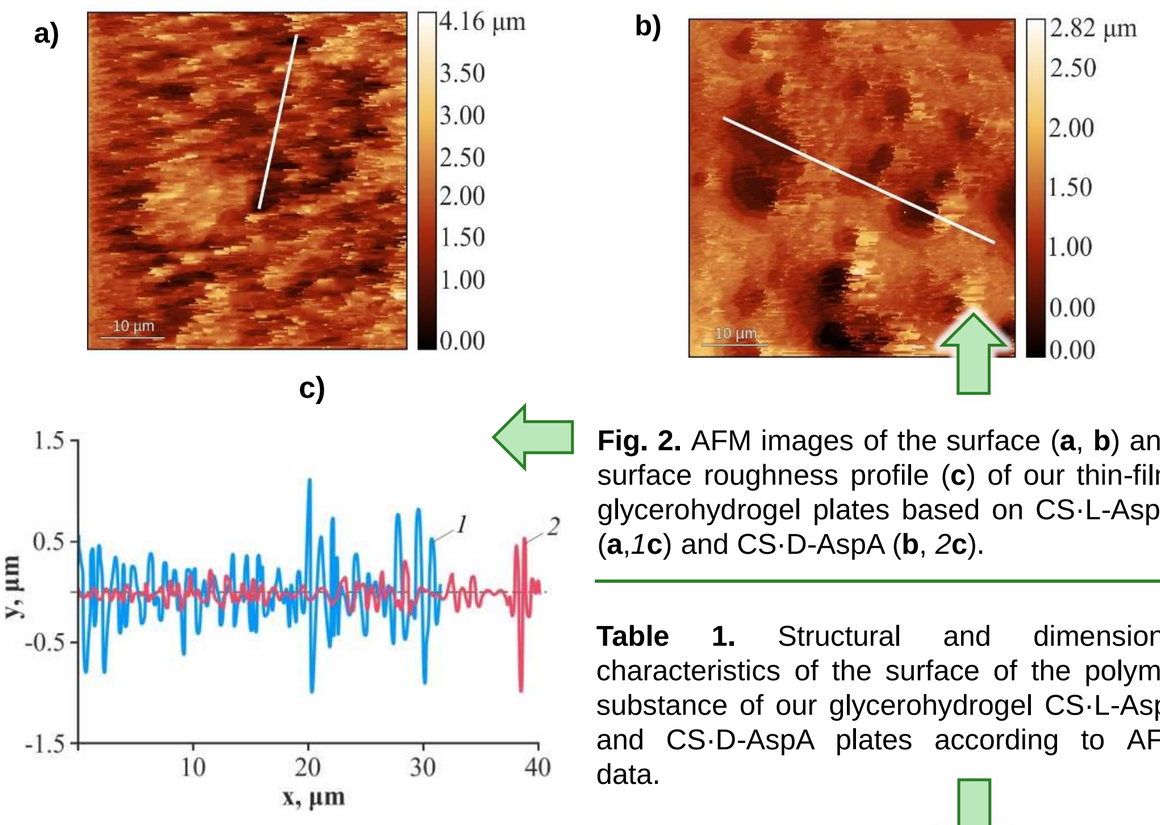

Fig. 2. AFM images of the surface (**a**, **b**) and surface roughness profile (**c**) of our thin-film glycerohydrogel plates based on CS·L-AspA (**a**,*1***c**) and CS·D-AspA (**b**, *2***c**).

Table 1. Structural and dimensional characteristics of the surface of the polymer substance of our glycerohydrogel CS·L-AspA and CS·D-AspA plates according to AFM data.

Parameter	Sample	
	CS·L-AspA	CS·D-AspA
Asymmetry R_{sk}	–0.02±0.39	0.09±0.7
Kurtosis R_{ku}	6±1	13±8

The solid phase isolated from the corresponding plates showed a more dense amorphous–crystalline ordering of the polymeric substance compared to the solid phase isolated from CS·L-(D-)AspA in the absence of silicon polyolate networks and a bioinert template.

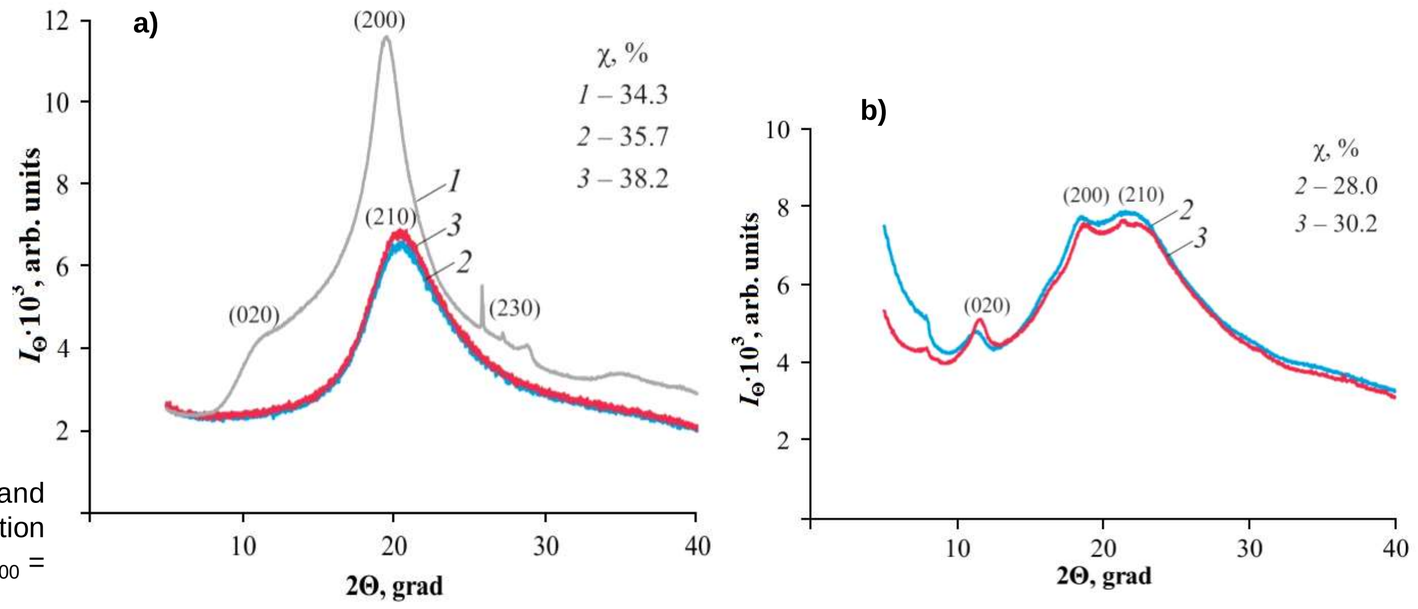

Fig. 3. X-ray diffraction patterns of the powders: initial sample of CS (*1***a**); CS·L-AspA (*2***a**) and CS·D-AspA (*3***a**), obtained from our thin-film glycerohydrogel plates; CS′·L-AspA (*2***b**) and CS′·D-AspA (*3***b**), obtained from solutions.

CONCLUSION

The revealed features allow us to consider our glycerohydrogel plates based on CS·L-(D-)AspA as promising biomimetic substrates to form tissue-engineered structures (including 3D bioprinting, electrospinning, supercritical fluid technologies, etc.) with a pregiven set of properties and accelerated growth of populations of epithelial and epitheliopod cell cultures.

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