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USE OF A BIOPRINTER WITH ALGINATE AS THE BIOINK

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Three-dimensional bioprinting aims to produce structures that mimic natural biological tissue using a bioink containing living cells and biomaterials, as the alginate which is commonly used in tissue engineering. The purpose of this research has been to compare the scaffolds of alginate hydrogels produced by bioprinting and manual deposition. In this study, a bioprinter OctuplusTM (3DBS - 3D Biotechnology Solutions) was used with human cells from the keratinocyte line (HaCat). Alginic acid sodium salt (3% alginate from Sigma-Aldrich) was solubilized in water, crosslinked with CaCl2. Previous results from our research group showed that this concentration of alginate hydrogels in water promoted better cell viability. The HaCat were directly cultivated at a density of 130,000 cells/well in 48 well tissue culture plates (control) or in cylindrical scaffolds composed of 3% (w/v) of alginate hydrogels or 3% alginate associated with 1.5% of gelatin. Cell viability was analyzed by MTT assay and cytotoxicity was tested by lactate dehydrogenase (LDH) assay. The MTT results showed that mean absorbance and the standard deviation (SD) after one day was 0.21±0.01 when the cells were cultivated directly in the culture plate (control); 0.16±0.02 when the bioprinter was used (not significant); 0.29±0.02 ($p<0.01$) when manual alginate deposition with syringe was used and 0.27±0.03 ($p<0.05$) when alginate with gelatin was used as a manual deposition. It was not possible to produce alginate scaffolds with gelatin on the bioprinter, possibly due to the higher viscosity of this mixture. In these results, the viability of the cell control group was similar to cell viability cultivated after bioprinting in alginate. The viability of cells in the scaffolds of alginate produced by bioprinting was worse than the viability of cells in the scaffolds produced by manual deposition. This decrease can be explained by the stress caused by the needle used in bioprinting. The comparison of the cell viability cultivated in alginate scaffolds (non-adherent hydrogel) produced by manual deposition with or without gelatin (contains cell adhesion sites) did not show statistical differences. The averages ± SD concentration of LDH released was 170±8, 139±8, 145±8 and 129±8 U/L, respectively to control, 3% alginate bioprinted, 3% alginate and 3% alginate + 1.5% gelatin, without significant statistical difference of the LDH leakage between the control and alginate groups (ps?0.05) differing from death control using 1% Triton X-100 ($p<0,01$). The LDH results demonstrated that alginate was not cytotoxic. Although alginate is a low-cost biomaterial and may be used as a bioink for bioprinting, high concentrations are difficult to solubilize. With the obtained concentrations, it has low viscosity if used as a bioink, since it deforms easily after extrusion. The results demonstrated that the use of bioprinting affected the viability of keratinocytes.