INTRODUCTION
Biomaterials are a fundamental component of tissue engineering to develop biological substitutes for the repair and regeneration of damaged tissues and organs. The purpose of the first and second phases was to determine and collect the physicochemical features of a series of chitosans in order to evaluate their effects on the secondary features of 3D chitosan scaffolds such as the water retention, and finally to select a suitable scaffold for developing a substrate for cell attachment and growth. A further goal was to enhance the skills related to design and development of 3D-printed structures. In the last part of the work, the main aims were to confirm cell attachment and follow the human cell growth on 3D-printed chitosan scaffolds.

EXPERIMENTAL METHODS
The 3D scaffolds were prepared by printing a chitosan solution [6% w/v in acetic acid 3.33 % (v/v) or lactic acid 1.48 % (v/v) or ascorbic acid 3.21 % (v/v)] a cooled surface (-18°C); then the scaffold was subsequently gelled in aqueous KOH (1.5M) or \( \text{NH}_3 \) or \( \text{Na}_2\text{CO}_3 \) (1.5M) for 1h. Normal dermal human fibroblast cells (Nhdf cells) and aneuploid immortal keratinocyte cells (HaCaT cells) obtained from American Type Culture Collection (ATCC®, Manassas, VA, USA) were used for in vitro tests performed up to 35 days and monitored using Neutral Red assays, scanning electron microscopy, histological analyses and MTT cell viability assays.

RESULTS AND DISCUSSION
The results obtained evidenced that several type of chitosans with different physicochemical features can be successfully used to create 3D structures using extrusion-based 3D printing process. 3D chitosan scaffolds fabricated from ascorbic acid solution showed excessive reactivity versus all gelling solutions, developing CO2 and weakening the structures, which were not useful to complete the experiment and collecting the data. In addition, 3D chitosan scaffolds fabricated from solutions with higher concentration of acid and degree of protonation are more hydrophilic. As for cell cultures, the best colonized scaffold were observed with HaCaT cells alone, and Nhdf and HaCaT cells together seeded on 3D chitosan scaffolds with a film of chitosan at the base and analyzed after 20 and 35 days. The cells were able not only to grow on the structures but also to fill the holes and to grow from the bottom to the top part of the scaffold. The results also showed and confirmed that the fibroblast cell growth was slower than keratinocyte cells.

CONCLUSION
Chitosan and its 3D structures afford good biological proprieties in terms of cytocompatibility and no toxicity toward two different skin associated human cell lines that successfully attach, grow and colonize the 3D chitosan scaffolds. These findings indicate that chitosan based 3D structures are promising candidate biomaterial for possible application in tissue engineering, to develop in vitro a 3D chitosan tissue.

REFERENCES
2. Smith G. et al., J. Biomech. 2:5-11, 2011