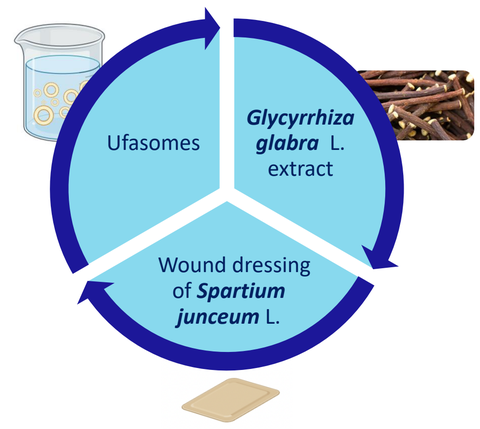
UFASOMES LOADED WITH *GLYCYRRHIZA GLABRA* EXTRACT FOR WOUND TREATMENT: PREPARATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

S. Rossello[a,b*], C. Cappadone[b], M. Mandrone[c], I. Chiocchio[c], M. Rossi[b], V. Sallustio[a], B. Luppi[a], A. Abruzzo[a], F. Bigucci[a], T. Cerchiara[a]

[a]Drug Delivery Research Lab., Department of Pharmacy and Biotechnology, *Alma Mater Studiorum*, University of Bologna, Via San Donato 19/2, Bologna, Italy.*simone.rossello2@unibo.it
[b]Pharmaceutical Biochemistry Lab., Department of Pharmacy and Biotechnology, *Alma Mater Studiorum*, University of Bologna, Via San Donato 19/2, Bologna, Italy.
[c]Pharmaceutical Botany Lab., Department of Pharmacy and Biotechnology, *Alma Mater Studiorum*, University of Bologna, Via Irnerio 42, Bologna, Italy.

Introduction

The appropriate management of wounds remains one of the major clinical challenges, as wound treatment now represents a significant cost to patients, the National Health System and the community. For this reason, current trends in wound care are moving toward the development of innovative wound products using natural herbals such as *Glycyrrhiza glabra* L., a perennial plant which grows spontaneously in Calabria and Abruzzo. Chemically, it mainly contains triterpene compounds and their derivatives, especially glycyrrhizin and flavonoids. Interestingly, anti-inflammatory, antioxidant, and antimicrobial activities have been detected in *G. glabra* extract rendering useful for wound treatment [1]. To preserve the biological activity extract, its encapsulation in nanosystems such as liposomes, niosomes, phytosomes, and ufasomes is considered a relevant strategy. Among the aforementioned nanovesicles, unsaturated fatty acid liposomes (Ufasomes) present advantages as good biocompatibility and easily available components [2].
The aim of this work was to encapsulate *G. glabra* extract into Ufasomes to combine the benefits of unsaturated fatty acids and *G. glabra* bioactive compounds promoting the wound healing process.



Preparation of Ufasomes

Ufasomes were prepared by mixing LipoidH90 (15 mg/mL), oleic acid (30 mg/mL) and linoleic acid (10 mg/mL) at 32°C until complete solubilization. The lipid mixture was homogenized at 9500 rpm for 2 minutes using a high-speed homogenizer (Ultra-Turrax T25 basic, IKA-werke). Subsequently, sonication treatment with a probe-sonicator (XL2020, Misonix Incorporated, Farmingdale, New York) for 1 cycle of 5 minutes was carry out. To achieve *G. glabra* extract –loaded Ufasomes (UfaGG), the lyophilized extract of *Glycyrrhiza glabra* L. (6 mg/mL) was solubilized in ultrapure water and add it, drop by drop, to the lipid mixture using a syringe. In Figure 1 is shown the preparation method of Ufasomes. Vesicle size, entrapment efficiency, zeta potential and *in vitro* release studies were investigated.

Figure 1. Preparation of *G. glabra* extract –loaded Ufasomes.

Ufasomes Characterization

- ✓ Unloaded Ufasomes and UfaGG showed a nanometric sizes.
- ✓ Formulations showed a low PDI value indicating the homogeneity of the vesicular systems.
- ✓ Zeta potential values of vesicles were negative and a value of zeta potential of ±30 mV indicates that the vesicles tend to repulse each other thus favoring system stability over time.
- ✓ *In vitro* release studies showed that after 6 hours about 70% of glycyrrhizin is released from the Ufasomes.

Table 1. VS, PDI, ζ potential and EE% of Ufasomes, unloaded and loaded with GG extract. The data are expressed as the mean of three replicate experiments ±SD.

	Size (nm)	PDI	ζ (mV)	EE%
Unloaded Ufasomes	219.06 ±19.88	0.223 ± 0.028	-30.24 ± 2.75	-
UfaGG	158.96 ± 3.60	0.168 ± 0.021	-30.16 ± 1.37	64.91 ± 3.27

Biocompatibility studies

The biocompatibility of GG extract, unloaded Ufasomes and UfaGG was evaluated on a human dermal fibroblast cell line (WS1) through a MTT assay. All tested concentrations of encapsulated extract (0.31% and 0.15% v/v) showed a high biocompatibility as no cell toxicity was observed (**Figure 2**)

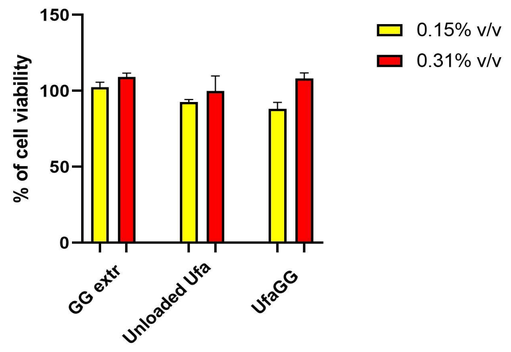


Figure 2. Ufasomes biocompatibility on a human dermal fibroblast cell line after 48 hours of treatment.

Intracellular ROS assay

To determine the antioxidant activity of Ufasomes, the levels of intracellular ROS were quantified. Through the use of TBH, oxidative stress was induced in human fibroblasts (WS1), after which the cells were treated 24 hours (with ascorbic acid AA, extract and nanovesicles). Finally, by means of the fluorescent probe DCFDA, the ROS were quantified. The results showed that UfaGG decrease oxidative stress by more than 50%.

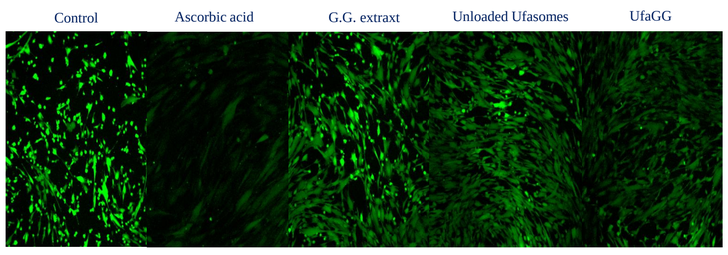


Figure 3. Images WS1 cells after staining with DCFDA probe. Images were acquired using a confocal microscope.

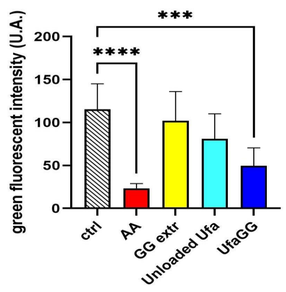


Figura 4. ROS levels quantification after treatment.

Biosafety

Biosafety was determined on human red blood cells (RBCs). Triton x100 was used as a positive control and PBS as a negative control. Various dilutions of Ufasomes in PBS were mixed with the RBCs and incubated for 30 minutes. Finally, the RBCs were centrifuged and the supernatant was read in the spectrophotometer. Haemolysis rate of less than 5% highlighted the formulation to be biosafe.
The results showed that *G. glabra* extract, unloaded Ufasomes and UfaGG were biosafe, showing a haemolysis rate less than 0.5%.

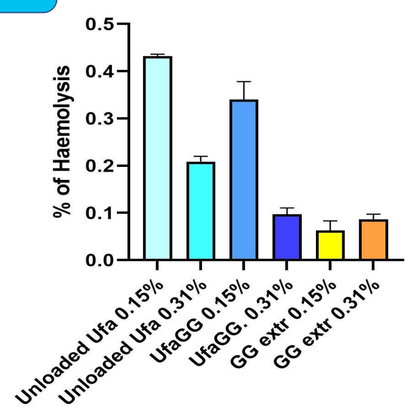


Figura 5. Heamolysis rate of *G. glabra* extract, unloaded Ufasomes and UfaGG.

Scratch test

To assess the speed of wound healing, a scratch test was carried out. Briefly, *G.glabra* extract, unloaded Ufasomes and UfaGG were applied on Spanish Broom gauze and, after a six-hour release in culture medium, its effect on cell proliferation and migration was assessed.

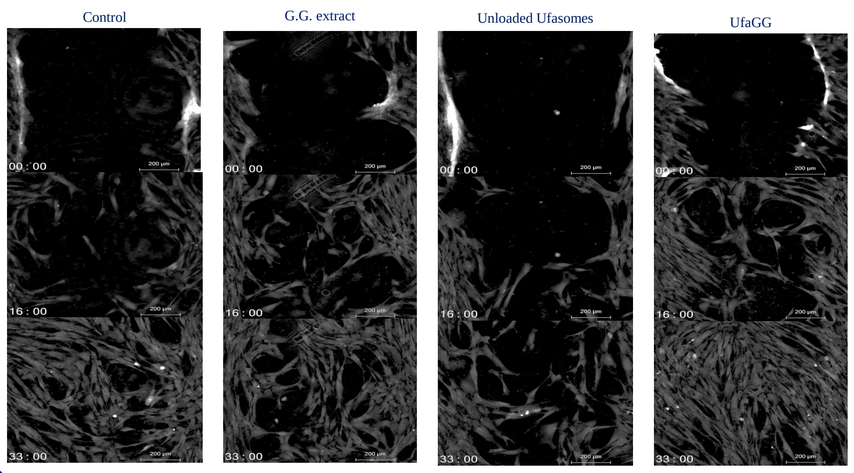


Figure 6. Images at different times (0, 16, 33 h) of the simulated scratch.

- ✓ The UfaGG lead to complete closure of the scratch after about 33 hours.

Conclusions

Glycyrrhiza glabra L. extract was successfully encapsulated into Ufasomes. UfaGG exhibited good physicochemical characteristics, such as nanometric size, a narrow PDI, an EE% of 64.91 ± 3.27%. In addition, biological studies revealed that UfaGG were not cytotoxic on human fibroblasts (WS1) and were biosafe. The ROS assay showed a reduction in oxidative stress in the fibroblasts treated with UfaGG. To conclude, the results showed that Ufasomes can be used as biocompatible nanocarriers for the encapsulation of *G. glabra* extract to improve the wound healing process.

References

[1] Vitale *et al.* Phytochemistry and Biological Activity of Medicinal Plants in Wound Healing: An Overview of Current Research. Molecules 2022, 27, 3566.
[2] Cristiano *et al.* Oleuropein-Loaded Ufasomes Improve the Nutraceutical Efficacy. Nanomaterials 2021, 11, 105.

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