

ADMAIORA

Grant Agreement number: 814413

Project acronym: ADMAIORA

Project title: ADvanced nanocomposite MAterIals fOr in situ treatment and
ulTRASound-mediated management of osteoarthritis

Funding scheme: H2020-NMBP-TR-IND-2018-2020

D4.2 Assembled innovative LIPUS stimulation set-ups

Due date of deliverable: [29/02/2020]

Actual submission date: [29/02/2020]

Start date of project: 01/01/2019

Duration: 49 months

Organisation name of lead contractor for this deliverable: [SSSA]

Deliverable author: [Irene Bernardeschi, Francesco Fontana, Andrea Cafarelli,
Leonardo ricotti]

Version: [4]

| Project co-funded by the European Commission within the H2020 Programme | | |
|---|--|----------|
| Dissemination Level | | |
| PU | Public | X |
| PP | Restricted to other programme participants (including the Commission Service) | |
| RE | Restricted to a group specified by the consortium (including the Commission Service) | |
| CO | Confidential, only for members of the consortium (including the Commission Service) | |

Document History

| Version | Date | Author | Summary of Main Changes |
|---------|------------|---|--|
| 1 | 13/02/2020 | Irene Bernardeschi, SSSA | First version of the template for project Deliverables |
| 2 | 27/02/2020 | Francesco Fontana, Andrea Cafarelli, SSSA | First draft of the Deliverable |
| 3 | 28/02/2020 | Leonardo Ricotti, SSSA | Revision of the Deliverable draft |
| 4 | 29/02/2020 | Irene Bernardeschi, SSSA | Final version of the Deliverable and submission |

Table of Contents

| | | |
|----------|--|----------|
| 1 | Executive summary | 4 |
| 2 | Assembled LIPUS stimulation systems | 5 |
| 2.1 | System 1 | 5 |
| 2.2 | System 2 | 6 |

| | | |
|----------|--------------------------|----------|
| 3 | Conclusions | 7 |
|----------|--------------------------|----------|

1 Executive summary

In this Deliverable, the highly controlled in vitro LIPUS stimulation prototypes are described. In section 2.1, both the low-frequency (38 kHz) and high frequency (500 kHz – 5 MHz) stimulation set-ups identified as SYSTEM 1 are presented. They will be used for ensuring the control of the US dose at the target and identifying (off-line) the optimum US parameters able to trigger beneficial bioeffects in terms of cartilage regeneration. In section 2.2, the assembled SYSTEM 2 set-up and its components is reported. It will allow investigating intracellular phenomena occurring during LIPUS stimulation in real-time.

Only few details on the two systems are reported in this Deliverable, which is public, to not jeopardise possible future IP protection. Full details on the different components have been reported in Deliverable D4.1. Furthermore, all additional and confidential information on the two systems is reported in Deliverable 4.3 (which is confidential).

2 Assembled LIPUS stimulation systems

In ADMAIORA, two different LIPUS set-ups have been designed. The former (**SYSTEM 1**) will ensure control of the US dose at the target for identifying (**off-line**) the optimum US parameters able to trigger beneficial bioeffects in terms of cartilage regeneration. The second one (**SYSTEM 2**) will allow to investigate intracellular phenomena occurring during LIPUS stimulation, in real-time (**on-line**) (Figure 1).

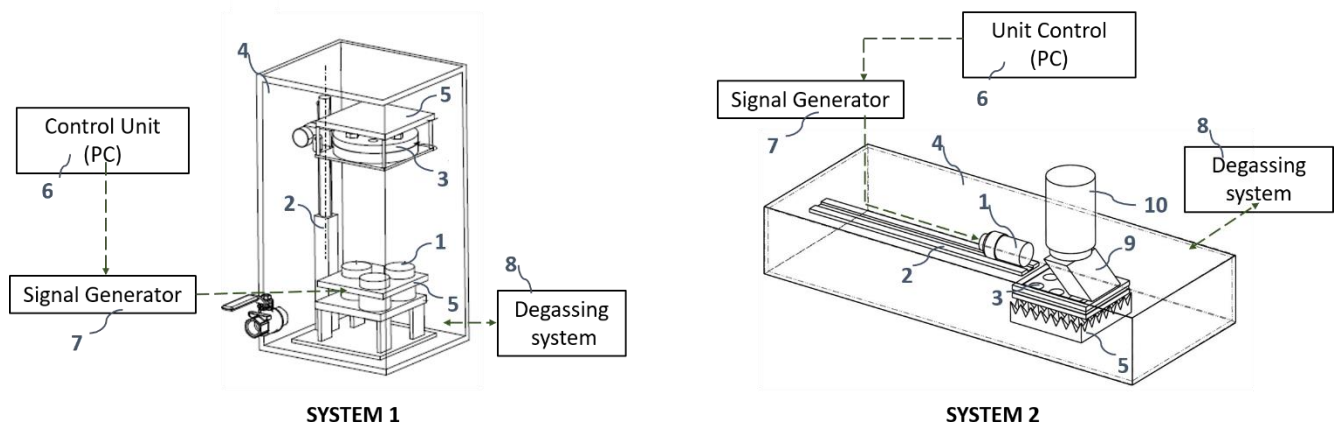


Figure 1. Design of the two innovative LIPUS stimulation systems targeted in ADMAIORA: **SYSTEM 1** (left) allows a multiple controlled US stimulation without reflections/attenuations; **SYSTEM 2** (right) allows a real-time optical visualization of the biological phenomena occurring *in vitro*.

2.1 System 1

The prototypes of the LIPUS systems for low (38 kHz) and high (500 kHz – 5 MHz) frequency and dedicated to cell stimulation have been assembled and are shown in Figure 2 and Figure 3, respectively.

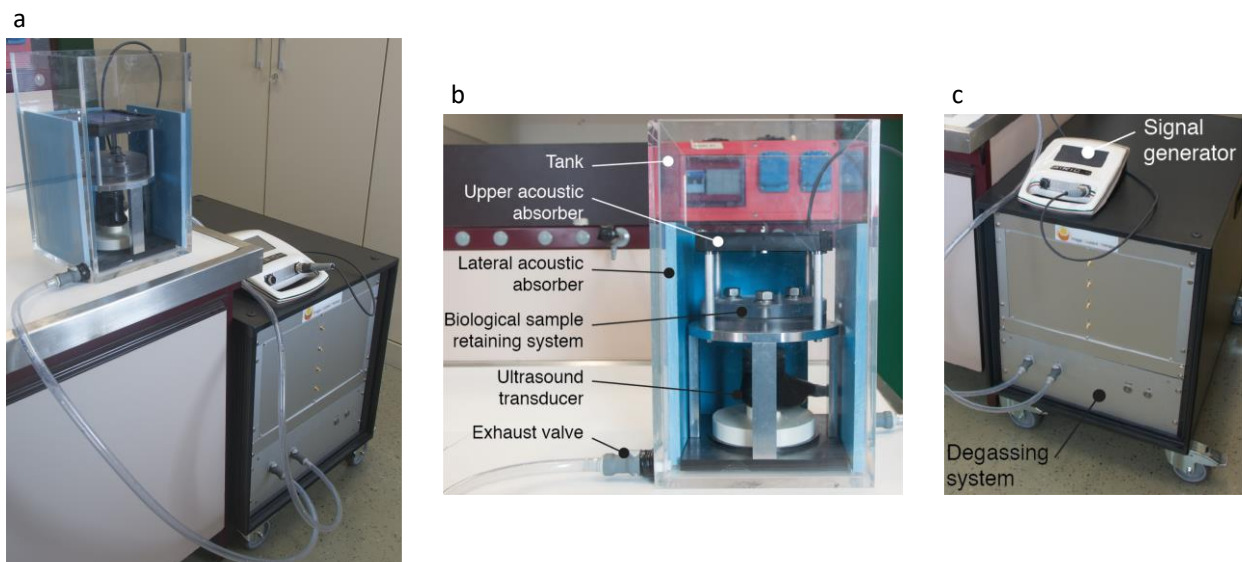


Figure 2. LIPUS system for low frequency (off-line) cell stimulation: (a) overall view; (b) detail of the tank and the internal components; (c) detail of the signal generator.

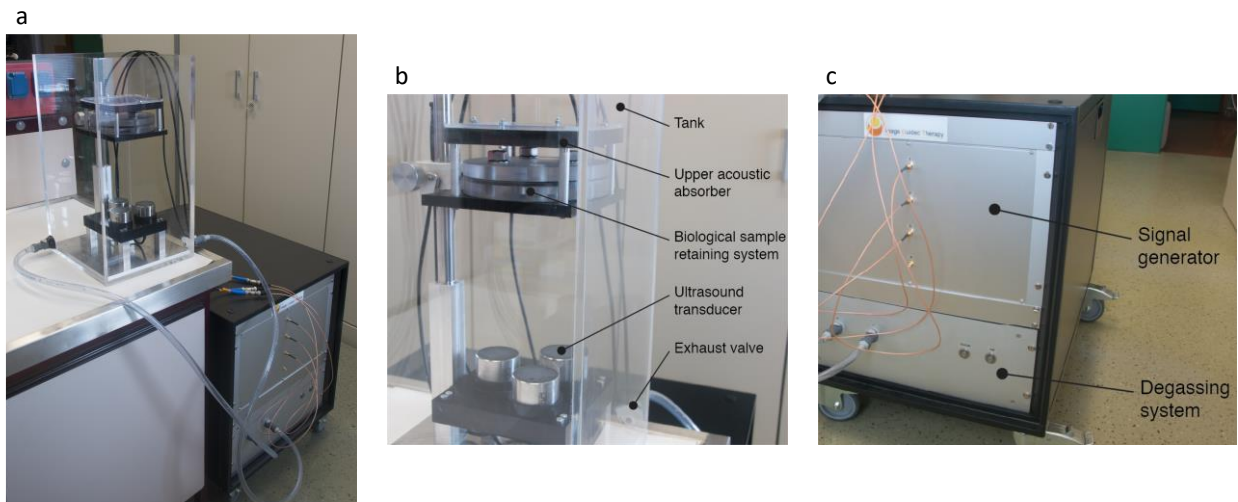


Figure 3. LIPUS system for high frequency (off-line) cell stimulation: (a) overall view; (b) detail of the tank and the internal components; (c) detail of the signal generator.

2.2 System 2

System 2 will guarantee a controlled US cell stimulation with simultaneous optical monitoring of the target. This feature can be interesting for a real-time investigation of US-triggered phenomena. The System 2 concept is protected by a patent that was part of the background of the ADMAIORA project¹.

The majority of the elements used to assemble System 1 (US transducers, retaining system, acoustic absorbers, signal generators etc.) were used also in System 2.

However, two key additional elements (*i.e.*, an opto-transparent/acousto-reflective element and an optical monitoring device) were added.

The assembled prototype of System 2 is shown in Figure 4. Figure 2

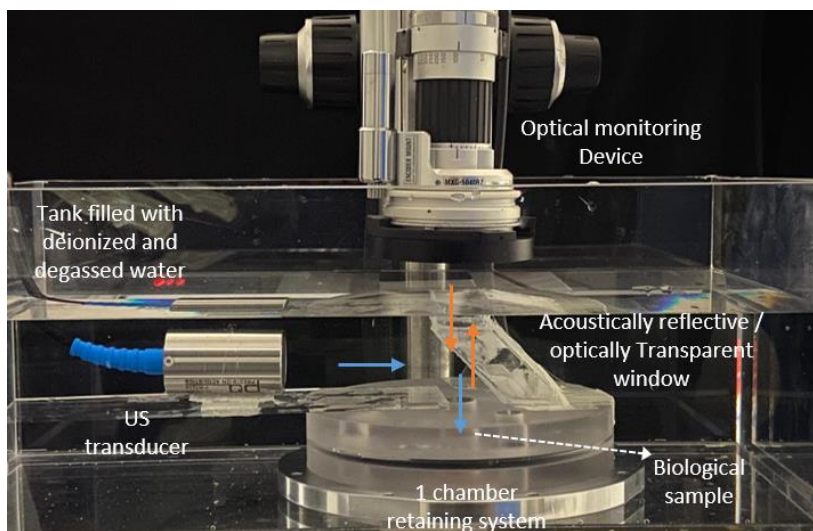


Figure 4. Image of System 2: LIPUS set-up that allows a controlled US cell stimulation with a simultaneous optical monitoring of the target (biological sample); blue arrows represent the US path, orange arrows represent the light path.

¹ Cafarelli et al. Italian Patent No. 102016000052583, Filing date: 23/05/2016

3 Conclusions

Two different LIPUS setups have been designed, built and assembled:

- **SYSTEM 1** for the release of US dose at the target (off-line) in a controlled way with consequent identification of optimal US parameters able to trigger beneficial bioeffects in terms of cartilage regeneration. In particular, two different set-ups have been developed for low frequency and high frequency US stimulation.
- **SYSTEM 2**, which allows to investigate intracellular phenomena occurring during LIPUS stimulation, in real-time (**on-line**).

In this Deliverable, a few pictures of the assembled prototypes are reported. No additional details have been provided, being a public Deliverable, to not jeopardise possible IP protection. A detailed description of each system component was already reported in Deliverable D4.1. All additional and confidential information on the two systems is reported in Deliverable 4.3 (which is confidential).