



WP 3.2: Engineering bioinspired and biomimetic nanomedicines for precise drug delivery

**Il ruolo della citometria a flusso nelle
valutazioni di Liposomi e Vescicole
Extracellulari come tools terapeutici**

**Università degli Studi di Urbino
23 maggio 2024**

**WP 3.2 Prof. Barbara Canonico
Dr. Mariele Montanari**





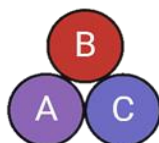
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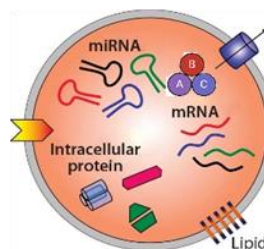
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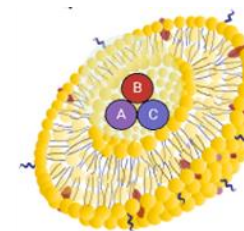
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***Campylobacter
jejuni* CDT**



**Evs from
infected cells
contain CDT**



***"Our"*
CDT-containing
Liposomes**



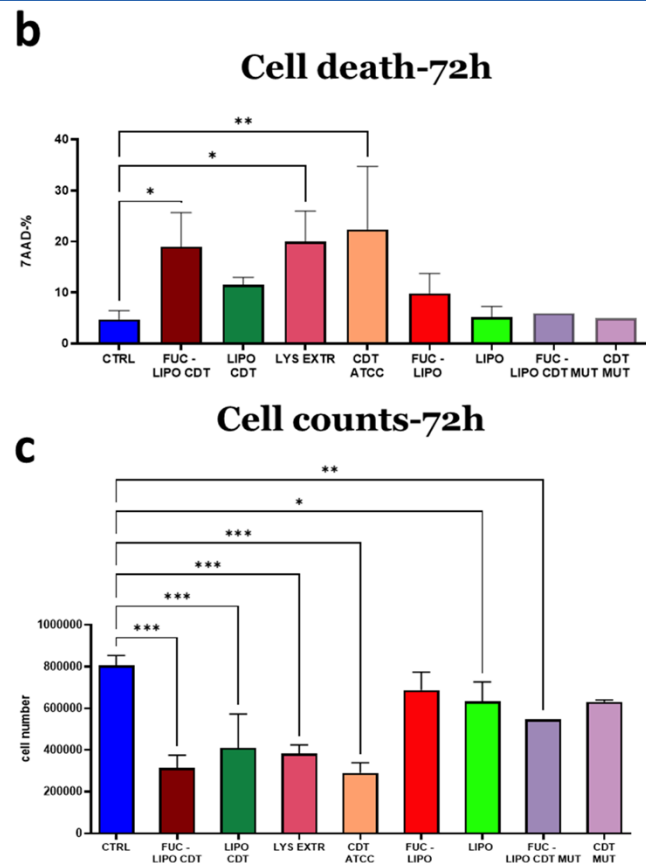
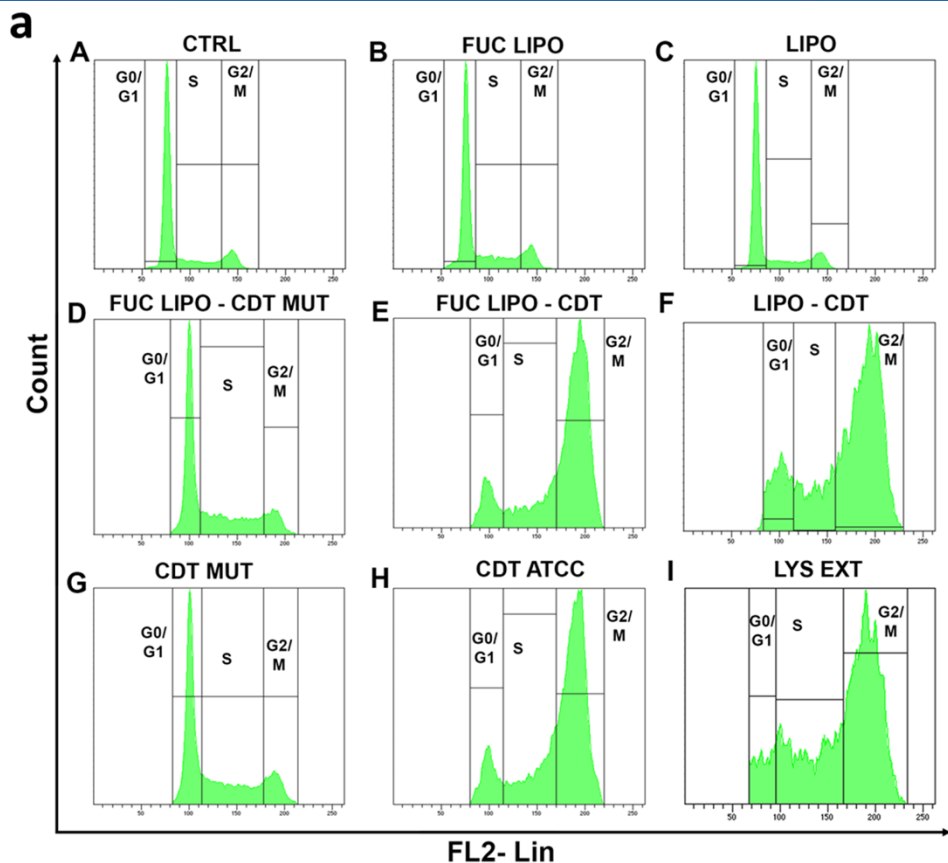
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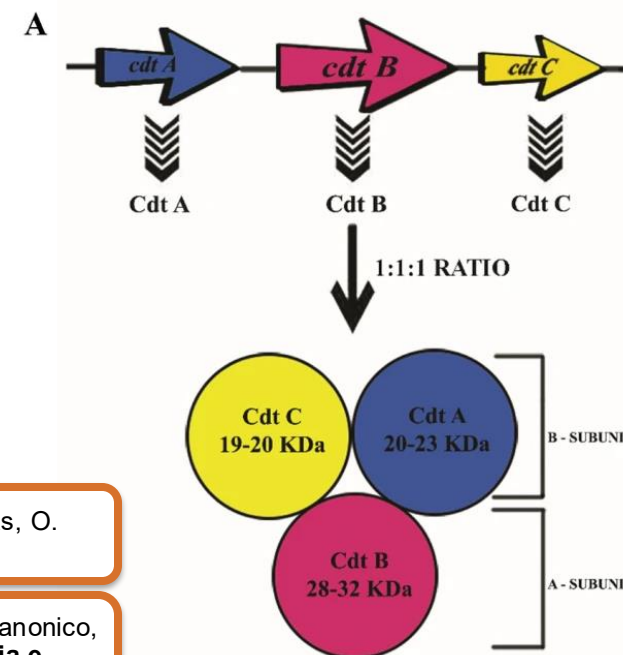
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Work in progress

CDT purificata ricombinante

Prof. Michele Menotta
Dott. Filippo Tasini
Dott. Gianluca Morganti



HETEROTRIMERIC HOLOTOXIN (AB₂ TYPE)

"C. Jejuni CDT-containing liposomes strongly inhibit proliferation in colon cancer cells." M. Montanari, M. Tiboni, G. Panza, D Lopez, F. Abbas, O. Gundogdu, M. Guescini, S. Papa, L. Casettari and B. Canonico. **CYTO 2024** (Edinburgo, May 3-9)

"Different lipid nanovesicle preparations containing C. jejuni CDT lysate differently inhibit proliferation in tumor intestinal epithelial cells". B. Canonico, M. Montanari, M. Tiboni, M. Guescini, D Lopez, G. Panza, L. Casettari and S. Papa. **76° Congresso Nazionale Società Italiana di Anatomia e Istologia Modena 2023**, (Sep 11-13)

Kailoo, Set al. 2021 World J Microbiol Biotechnol
<https://doi.org/10.1007/s11274-021-03117-z>



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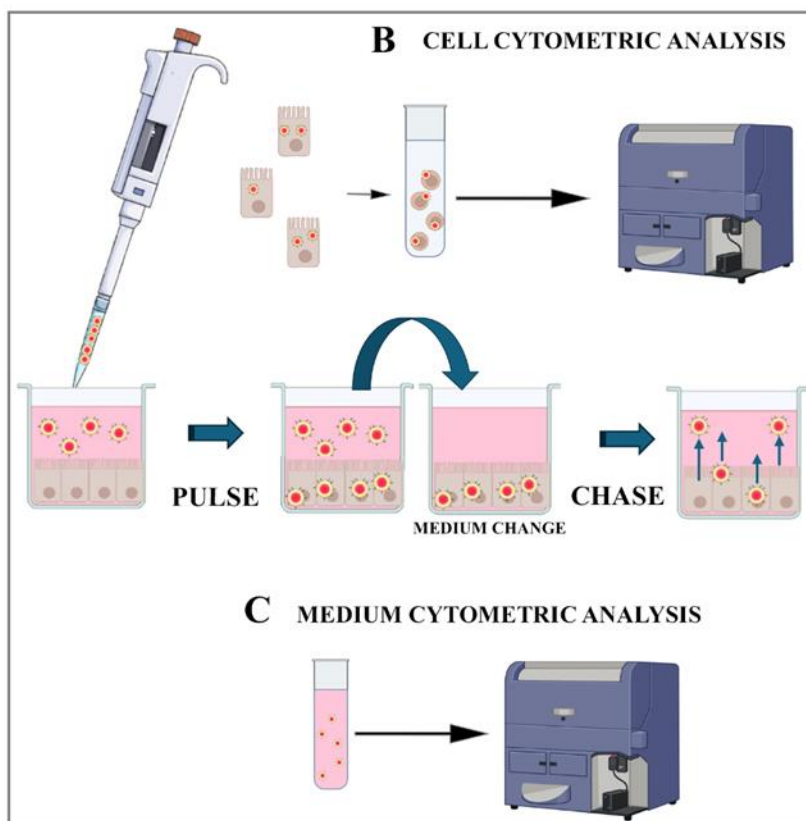
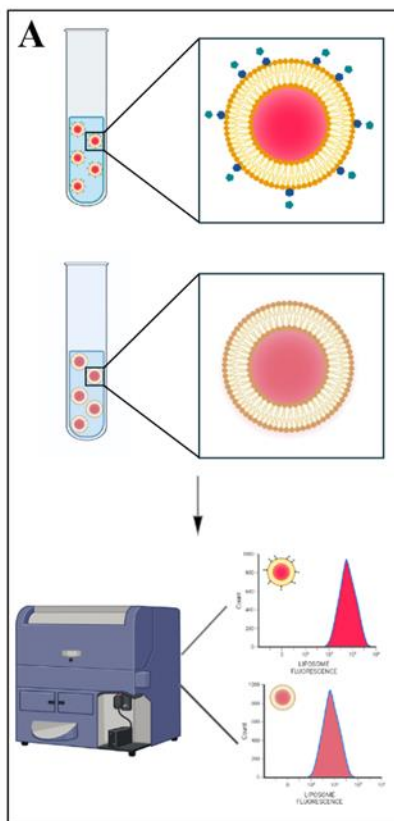
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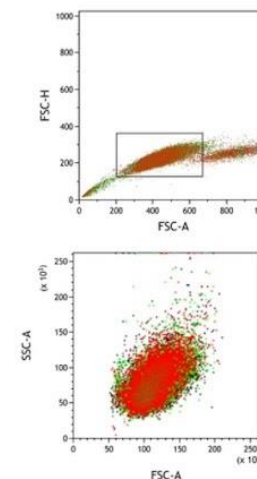
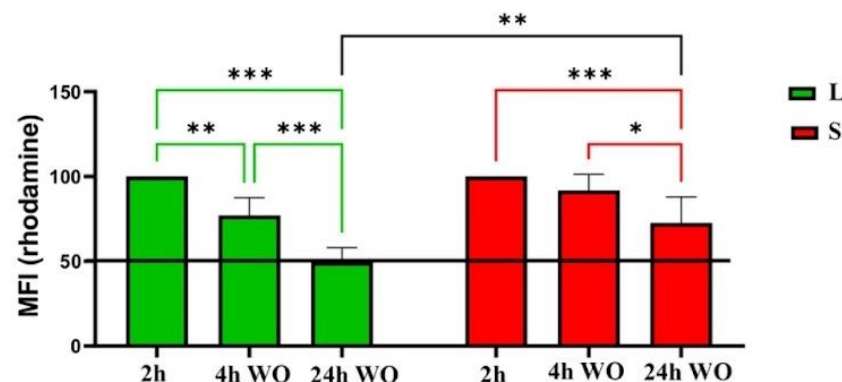
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OTTIMIZZAZIONE del PROTOCOLLO

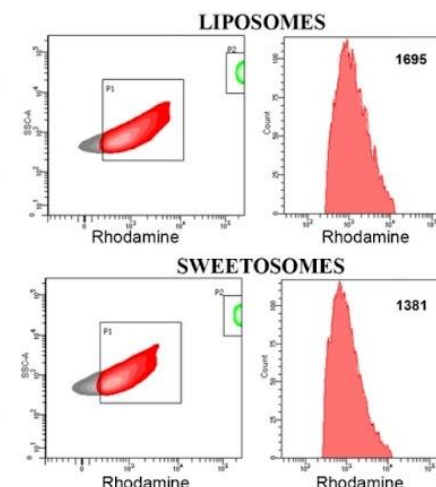
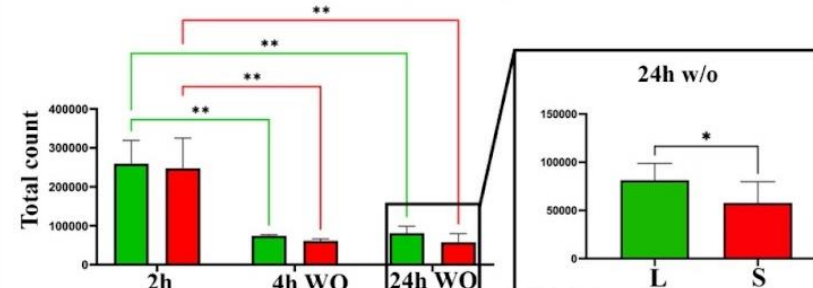
FIRST PART OF METHOD



PULSE AND CHASE LIPOSOME UPTAKE HT-29 CELLS



RHODAMINE-LIPOSOME COUNT (in the culture medium) HT-29 cells



Prof. Luca Casettari,
Dott. Mattia Tiboni

"Selective Endocytic Uptake of different decorated Liposomes in intestinal cancer cells" Canonico Barbara, Montanari Mariele, Tiboni Mattia, Lopez Daniele, Panza Giovanna, Casettari Luca and Papa Stefano. **XXIV Convegno Ed Assemblée Collegio Dei Docenti Di Istologia Ed Embriologia Umana**, Napoli (feb 20-21, 2025)



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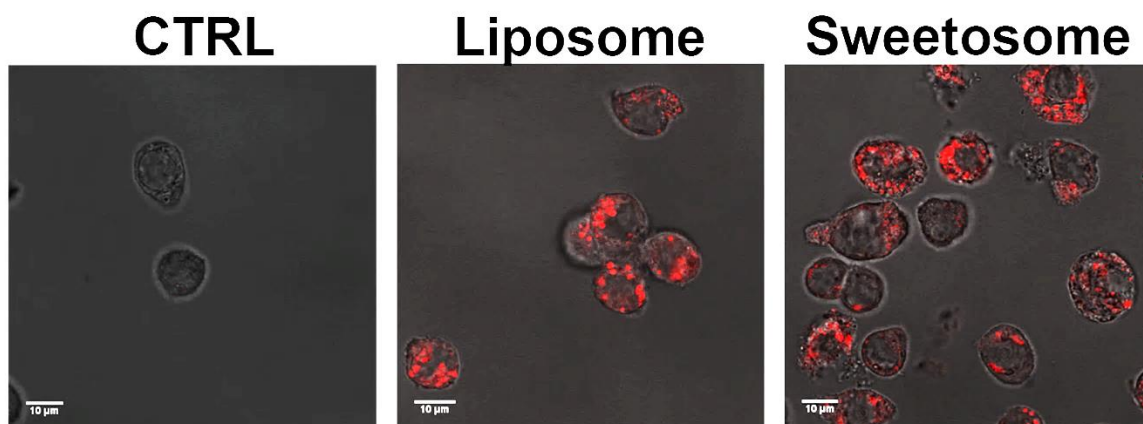


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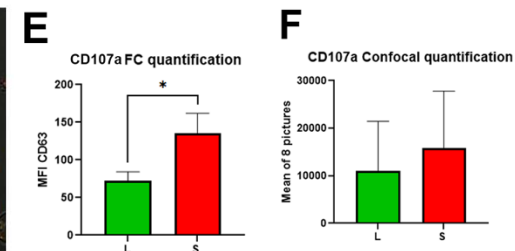
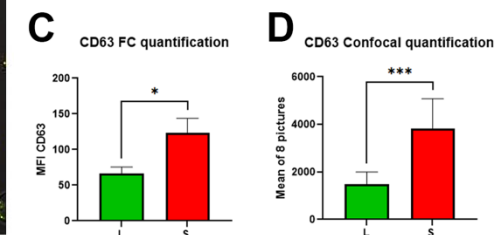
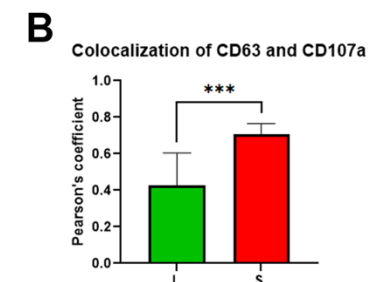
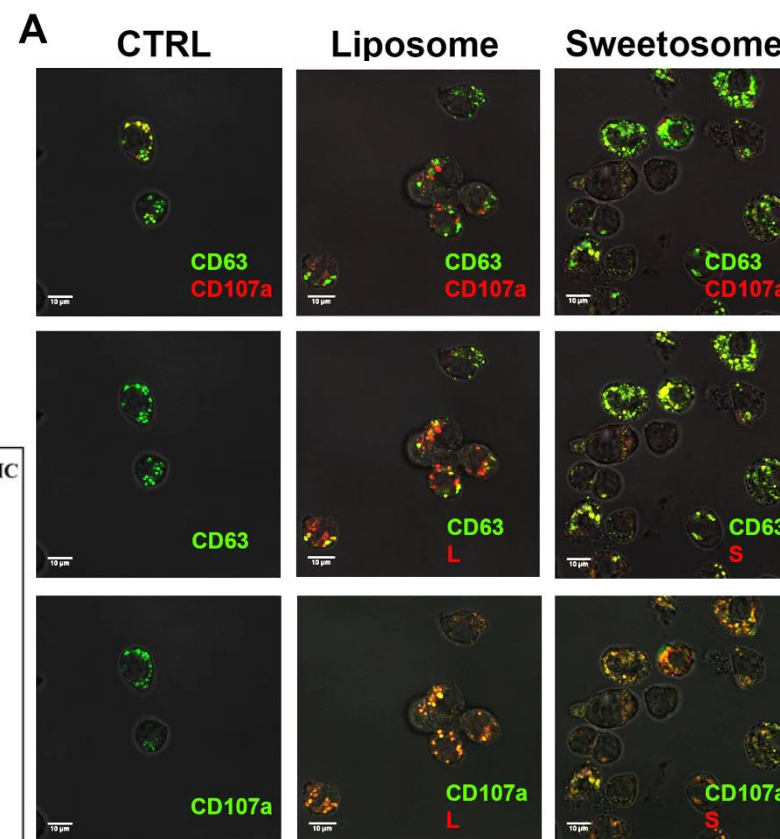
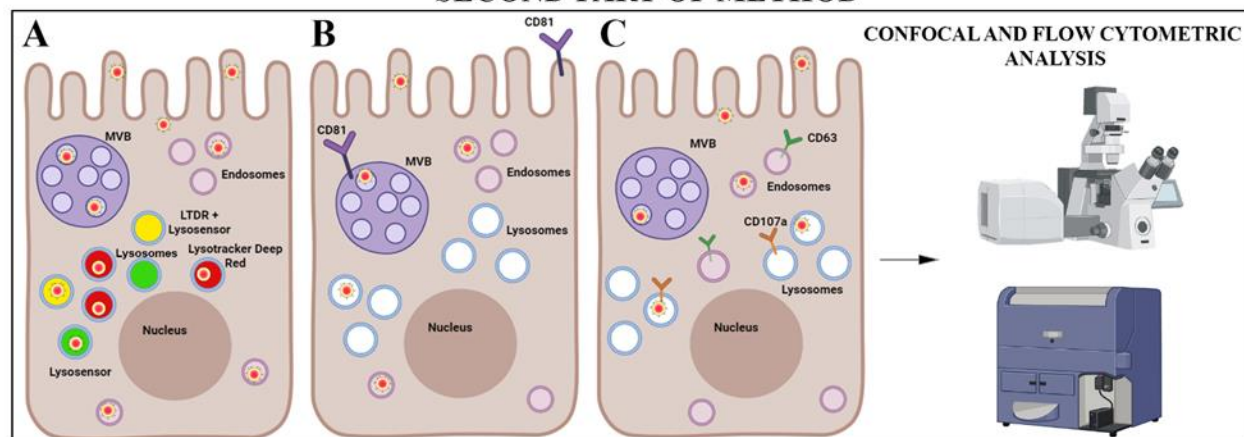


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DESTINO INTRACELLULARE: PERCORSO ENDO-LISOSOMIALE



SECOND PART OF METHOD





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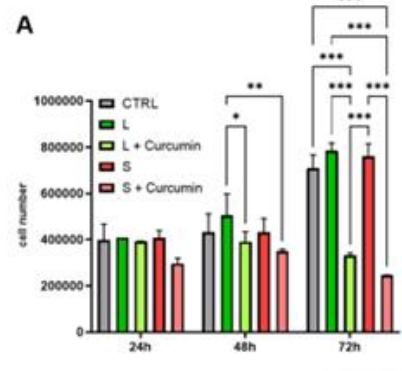
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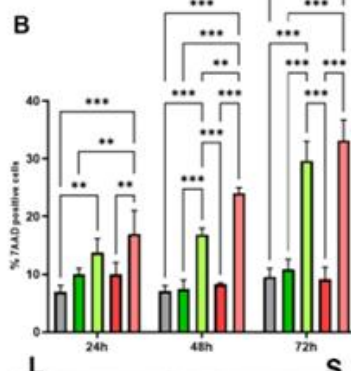
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FORMULAZIONI LIPOSOMIALI CONTENENTI CURCUMINA

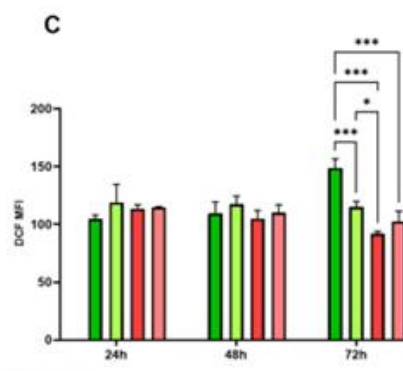
Inhibition of Cell Proliferation
on Caco-2 cells



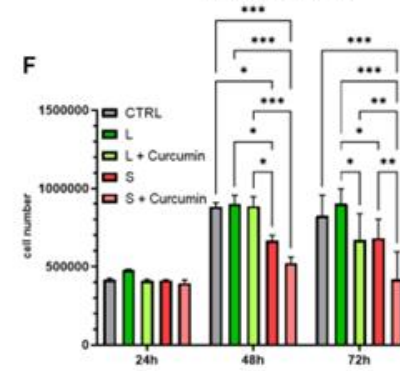
Cell death on Caco-2 cells



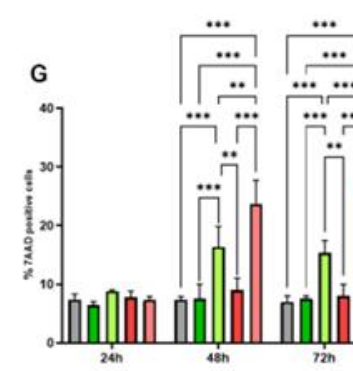
Hydrogen peroxide
content on Caco-2 cells



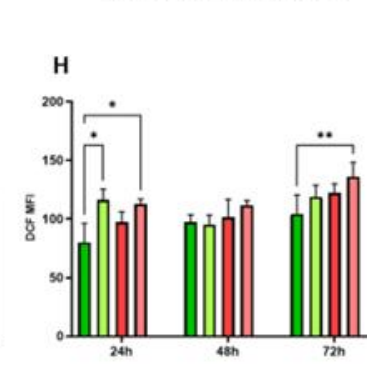
Inhibition of Cell Proliferation
on HT-29 cells



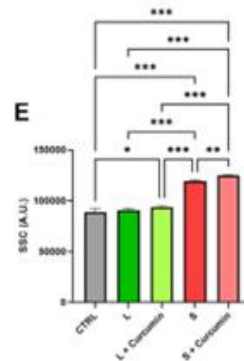
Cell death on HT-29 cells



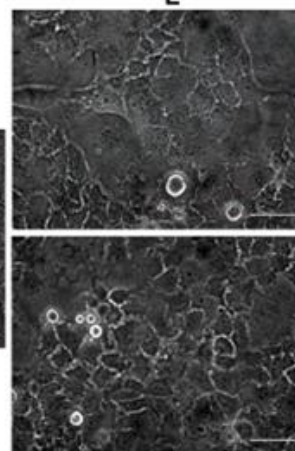
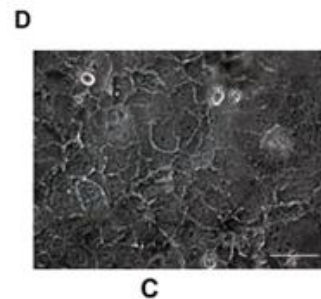
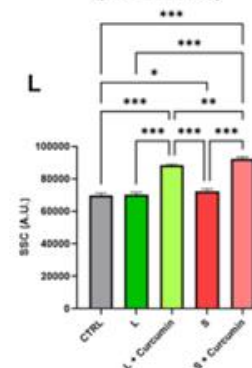
Hydrogen peroxide
content on HT-29 cells



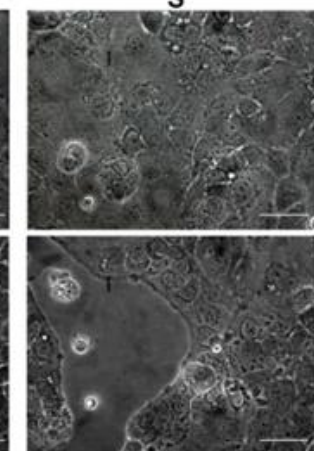
Intracellular Granularity
(24h Caco-2)



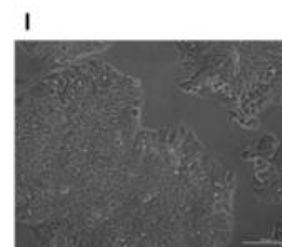
Intracellular Granularity
(24h HT-29)



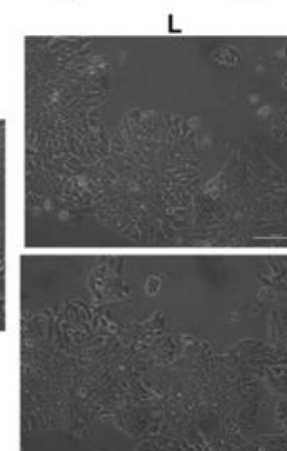
L + Curcumin



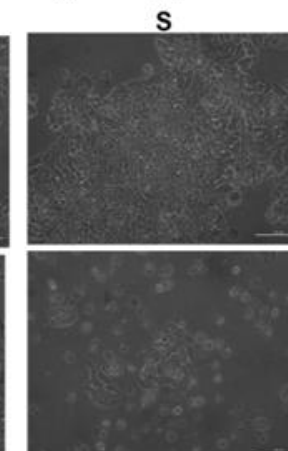
S + Curcumin



C



L + Curcumin



S + Curcumin



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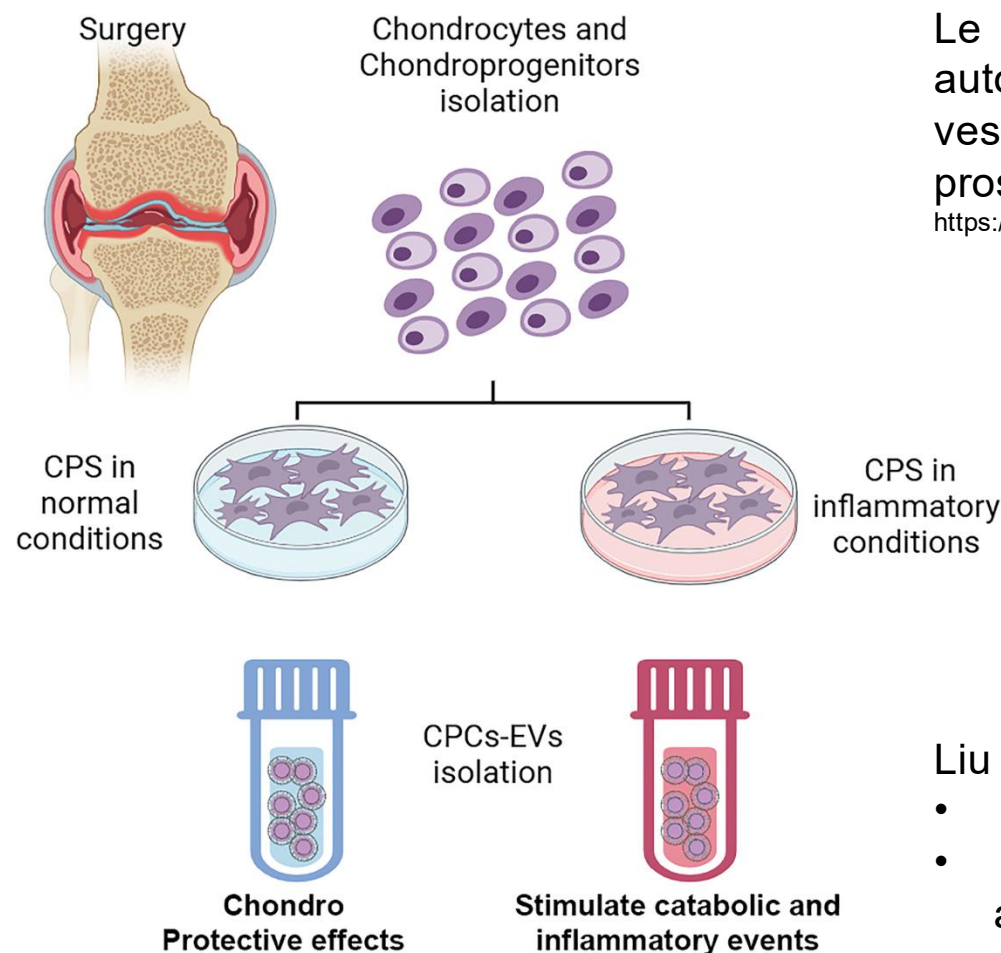


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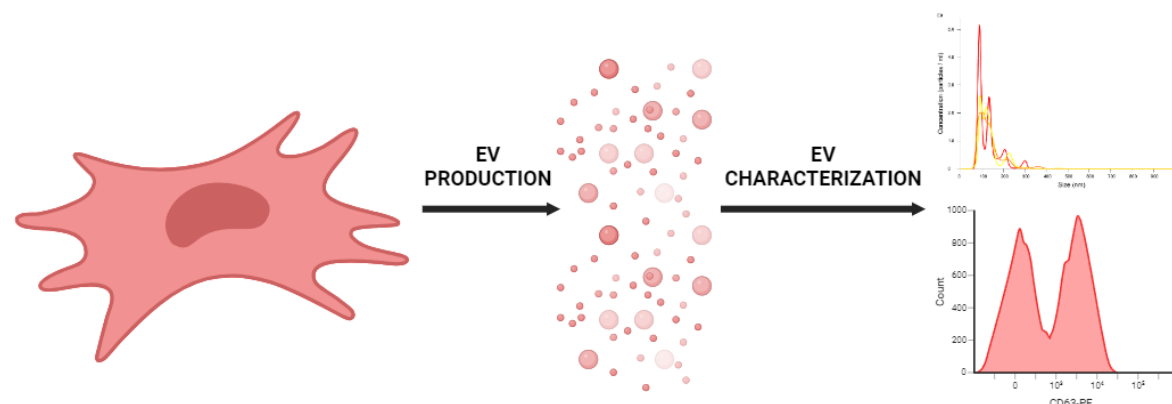


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EVs da cellule progenitrici condrogeniche



Le cellule progenitrici condrogeniche (CPC) hanno un'elevata capacità di autorinnovamento e potenziale condrogenico. Per cui ad oggi, oltre alle vescicole extracellulari rilasciate dalle MSC per il trattamento dell'OA, viene prospettato l'utilizzo di Evs da CPCs. (Wang, R., et al. 2020 *Stem Cell Res Ther.* <https://doi.org/10.1186/s13287-020-01594-x>, Feng, K., et al. 2024 *J Nanobiotechnol.* <https://doi.org/10.1186/s12951-024-02632-z>)



Liu et al 2020 hanno riscontrato che le EVs rilasciate da condrociti coltivati:

- in un **ambiente normale** hanno avuto un effetto protettivo sui condrociti,
- in un **ambiente infiammatorio** hanno stimolato eventi catabolici nei condrociti articolari. (Liu, X et al 2020 *Journal of orthopaedic research.* <https://doi.org/10.1002/jor.24525>)



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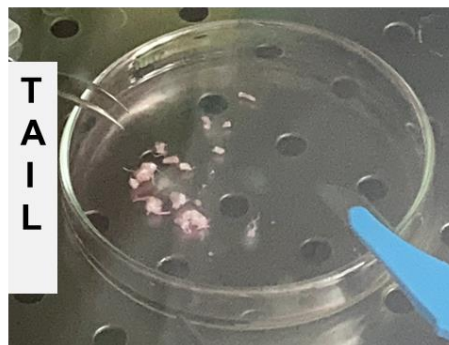


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APPROVAZIONE COMITATO ETICO del progetto:

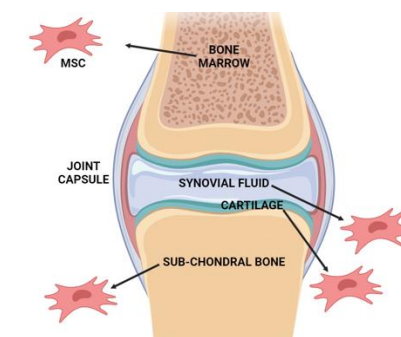
“Disgregazione di tessuti connettivi
(cartilagine e osso) per l’ottenimento
e caratterizzazione funzionale delle
cellule (tra cui le
staminali/mesenchimali) e delle loro
vescicole extracellulari”

nella seduta n. 84 del 25 luglio
2024.



Articular
Cartilage

Articular Cartilage
minced



Collagenase procedure



Mechanical Automated procedure





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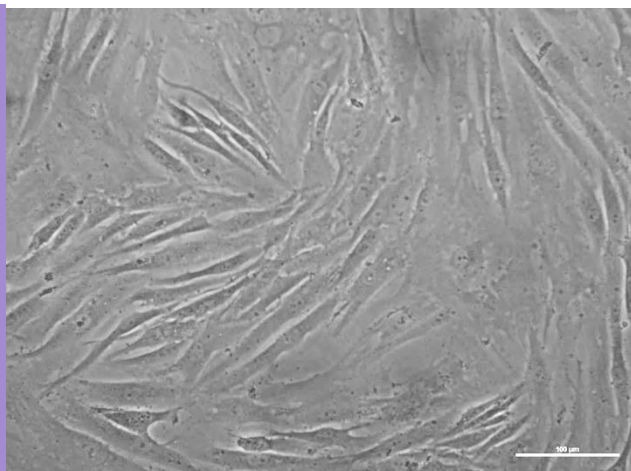


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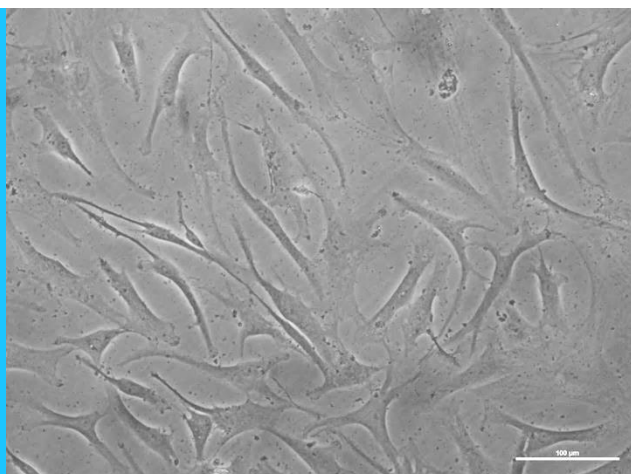


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16h Collagenasi



Medi+Coll 4h

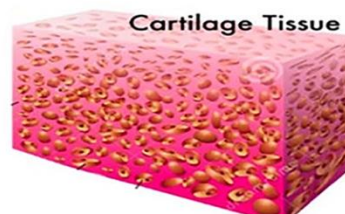


Tsoukas, D., et al.
2023. *Bioengineering*.
<https://doi.org/10.3390/biengineering10111294>

Non-Arthroscopic Autologous Cartilage Micrografting

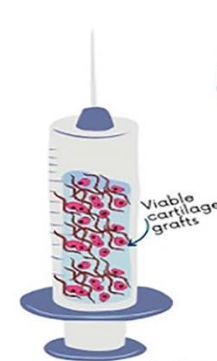
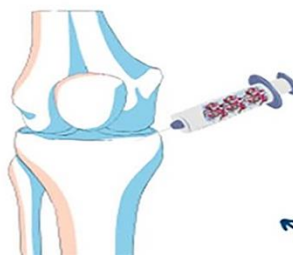


Mechanical disaggregation performed
using the Rigenera® technology to
obtain the Autologous Micrografts
(AMT®) cartilage suspension



Autologous cartilage tissue
with perichondrium

Infiltration of the joint with the
Autologous Micrografts (AMT®)
cartilage suspension



Injectable Autologous Micrografts (AMT®)
cartilage suspension

Feng et al. *Journal of Nanobiotechnology* (2024) 22:345
<https://doi.org/10.1186/s12951-024-02632-z>

Journal of Nanobiotechnology

RESEARCH

Open Access

Cartilage progenitor cells derived
extracellular vesicles-based cell-free strategy
for osteoarthritis treatment by efficient
inflammation inhibition and extracellular
matrix homeostasis restoration

Kai Feng^{1†}, Feng Wang^{1†}, Hongfang Chen^{2†}, Rui Zhang¹, Jiashuo Liu¹, Xiaodong Li³, Xuetao Xie^{1*} and Qinglin Kang^{1*}



Valutazione dell'impatto delle procedure disgregative (meccaniche, enzimatiche o combinazione delle 2) sulla quantità e qualità delle EVs prodotte da CPCs in coltura, nell'ottica di possibile applicazione autologa.



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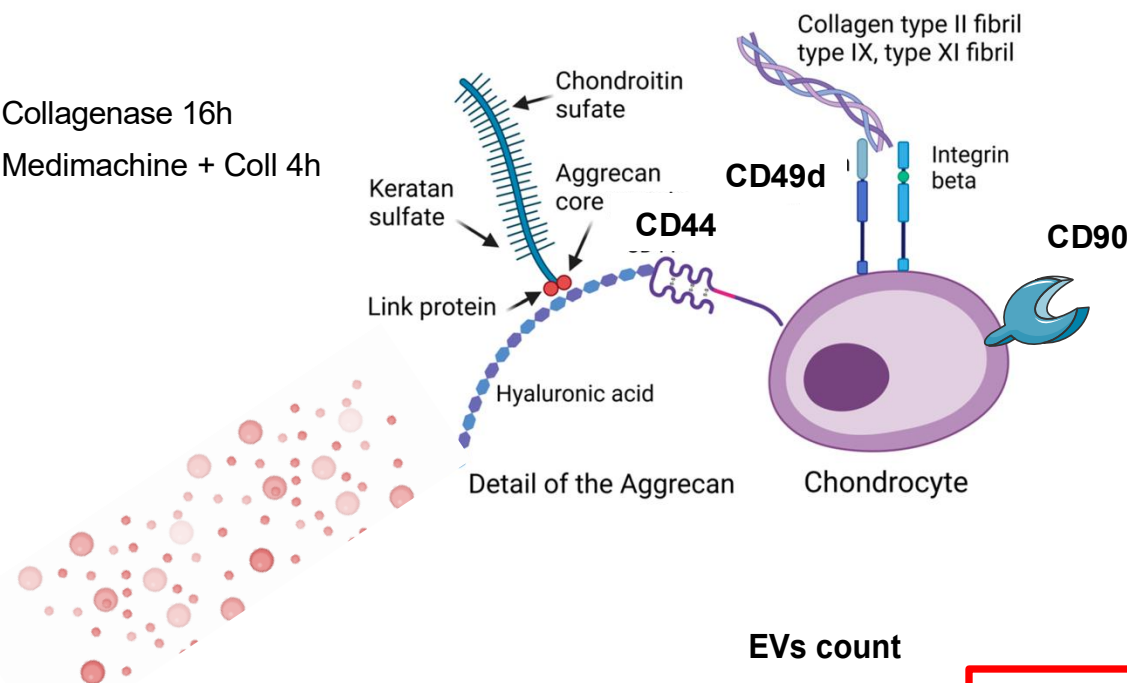
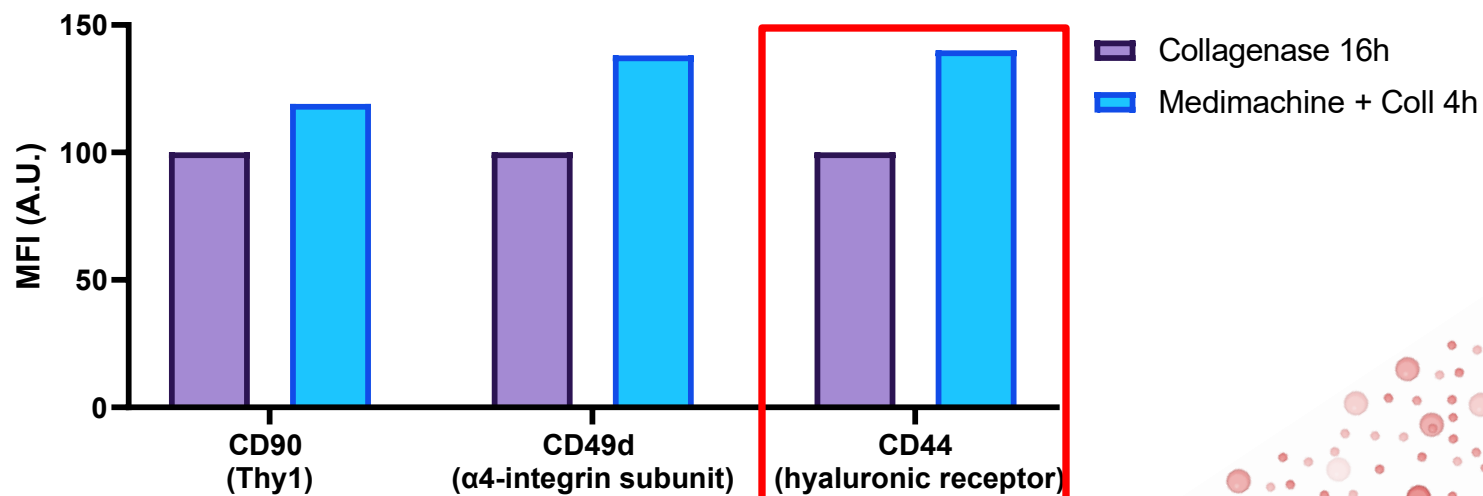


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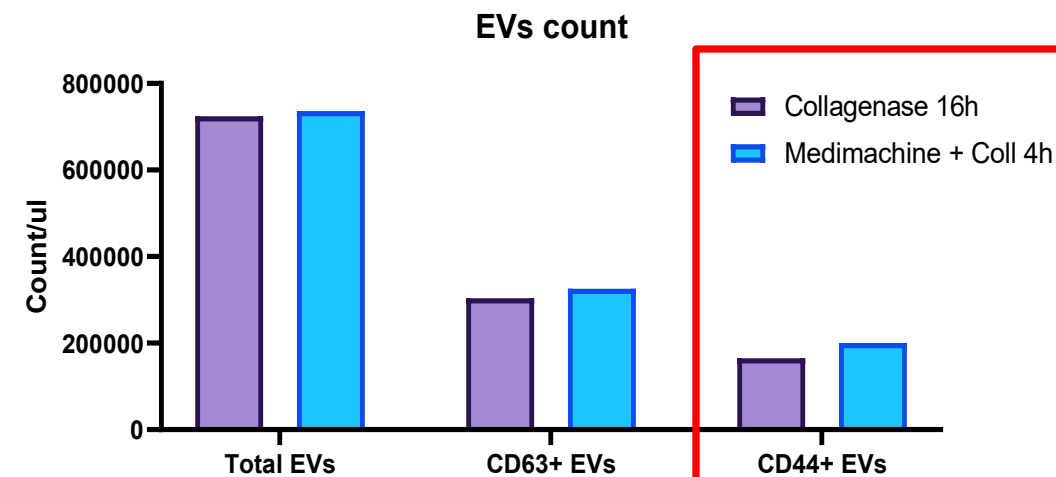
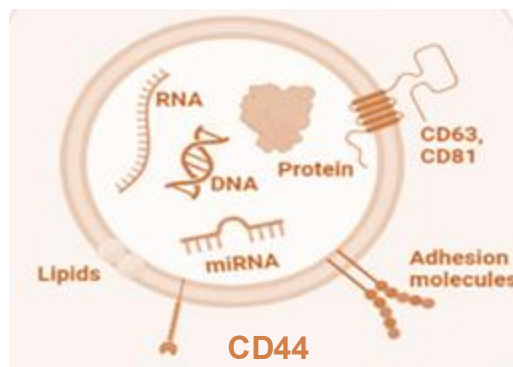
Cell surface expression of stem cells markers



Modificata da Bačenkova, D., et al. 2023
<https://doi.org/10.3390/jms242317096>

DATI PRELIMINARI

WORK IN PROGRESS





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Acknowledgements

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Dott. Diego Ghinelli
Dott. Alessandro Beccarini

GRAZIE
PER
L'ATTENZIONE