Institute of Biochemistry, Bucharest

LIPOSOMES, FROM PROPERTIES TO PERFORMANCE

The achievement of efficient drug delivery system in the treatment of inflammatory disorders

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Interest to use lipid nanostructures (liposomes) as drug delivery system

- **One of the major interest in the Nanomedicine area** is the development of efficient nanosized drug delivery systems specialized for intracellular delivery of macromolecular therapeutics able to increase the drug specificity and selectivity and minimize the side effects.

- Due to their composition and biocompatibility, the lipid nanostructures (liposomes) have real qualities which enable them to function as efficient transporters in the process of controlled drug release.

- Our group from Institute of Biochemistry developed studies in using liposome as drug delivery system in the frame of national and international projects.

Background

Lipid nanocapsules / Liposomes are vesicular structures composed of one or more phospholipid bilayer membranes separated by aqueous spaces

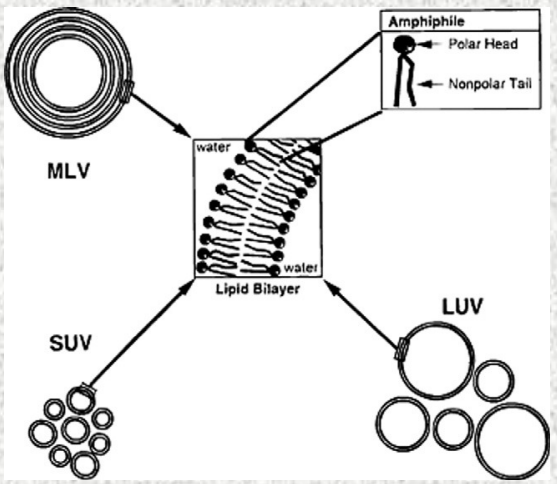
Essential physical and chemical parameters:

- **lipid composition of membranes**
- **size**
- **surface electrical charge**

Distinct types of liposomes can be classified:

By number of bi-layers:

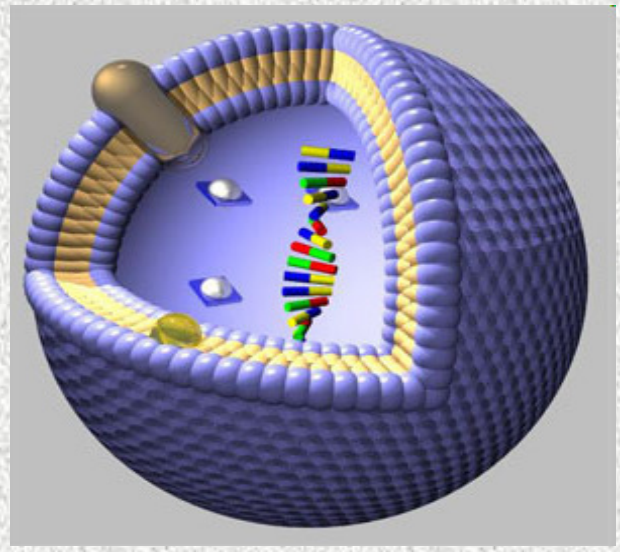
- ***Unilamelar***
- ***Oligolamelar***
- ***Multilamelar***



By size:

- **Small · <150 nm in diameter (SUV)**
- **Large · >150 nm in diameter (LUV)**

SUV are lipid nanostructures also known as nanosomes (Castor TP, *Current Drug Delivery*, 2005)



Avanti Polar Lipids; www.avantilipids.com

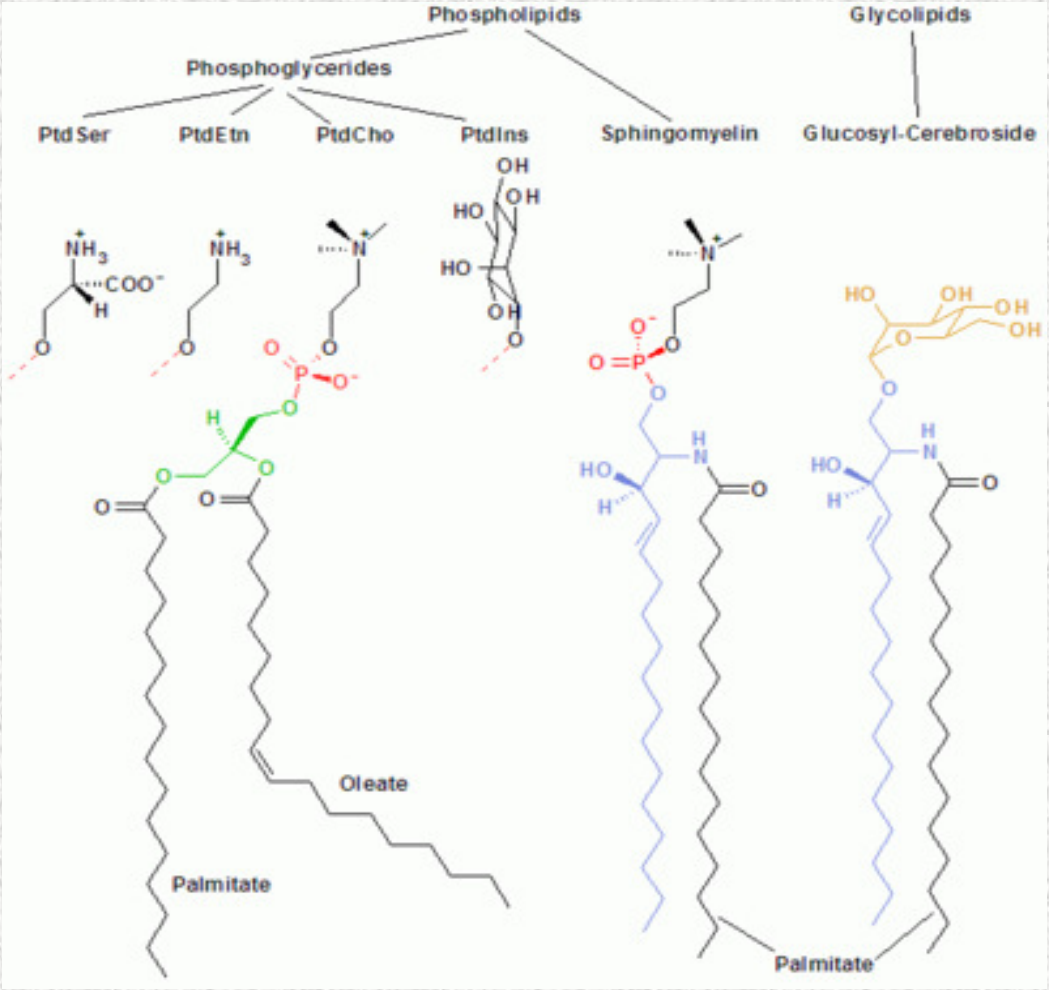
Characteristics

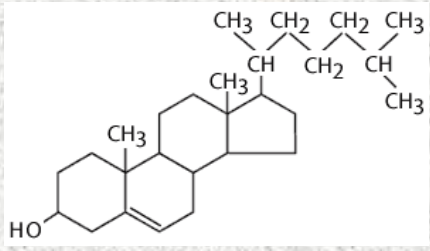
- prepared from natural, biodegradable and nontoxic lipids
- **able to entrap hydrophilic drugs in the large aqueous interior and lipophilic drugs inserted in the lipid bilayer.**
- good candidates for targetting of therapeutic agents to the site of interest

Advantages of using liposomes as drug carriers

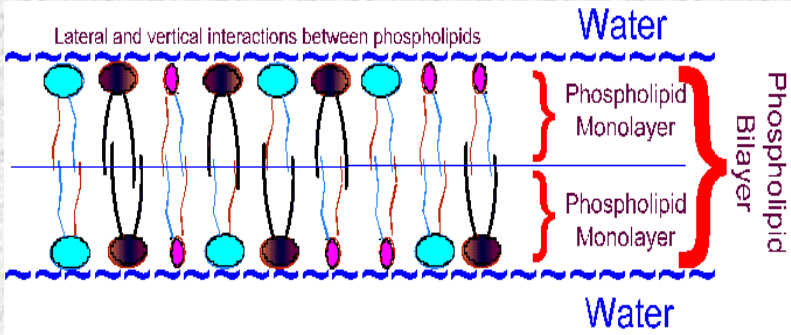
- ***Protection and efficiency***	- Liposome encapsulated drugs are inaccessible to metabolising enzymes - The same therapeutic effect is obtained with a lower drug concentration
- ***Duration of action***	- Liposome can prolong drug action by slowly and controlled release of the encapsulated molecules in the body (drug depot)
- ***Directing potential***	-Targeting options change the distribution of the drug in the body
- ***Solubilisation***	-Liposome may solubilise lipophilic drugs
- ***Amplification***	-Liposome can be used as adjuvants in vaccine formulations
- ***Internalisation***	- Liposome are endocytosed by cells being able to deliver the encapsulated material into the cell. Liposome are also able to bring plasmid material into the cell throgh the same mechanism (non viral transfection system)

The major membrane lipids used for liposome





Cholesterol is a sterol (a combination of steroid and alcohol) and lipid



Phospholipids:
PtdCho - Phosphatidylcholine; **PtdEtn** - Phosphatidylethanolamine; **PtdIns** - Phosphatidylinositol; **PtdSer** - Phosphatidylserine.

Our Research Achievements

1. **Preparation and characterization of liposomal systems containing**

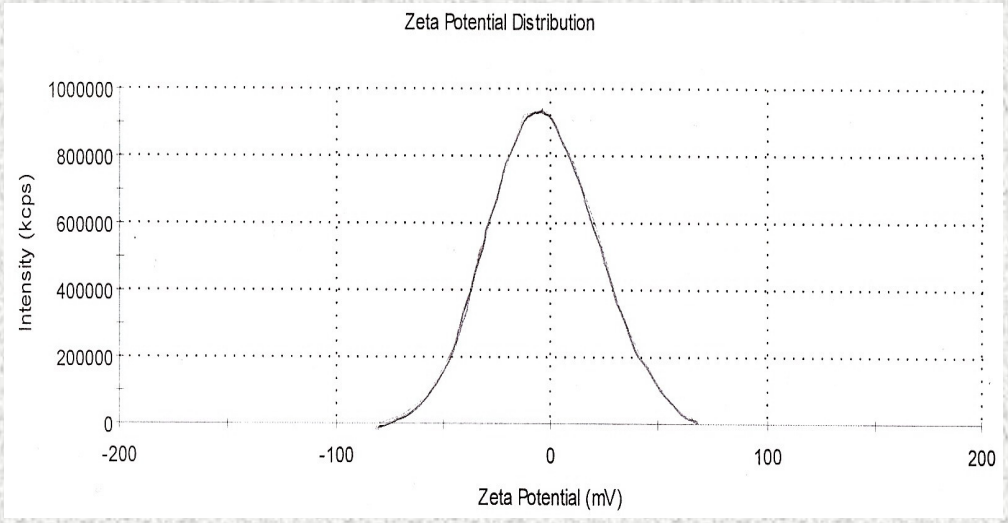
   - **hydrophilic bio-active molecules in the aqueous interior**

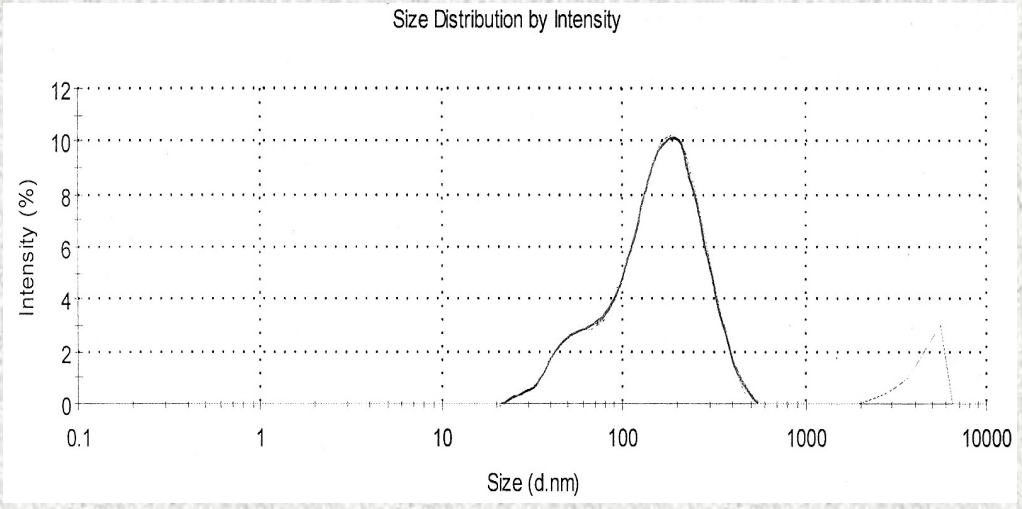
   - **lipophilic drugs inserted in the lipid bilayer**

2. **Biocompatibility tests in cell culture**

3. **Anti-inflammatory activity of liposome systems**

Extrusion technique to generate vesicles of controlled size





liposomal population,liposome diameter ~ 157 nm (Dinamic Light Scattering using **Malvern Autosizer**)

Mini-Extruder from Avanti Polar Lipids

The particle size distribution of vesicles prepared by extrusion is a function of the number of passes through the polycarbonate membrane of the hydrated lipid suspension.

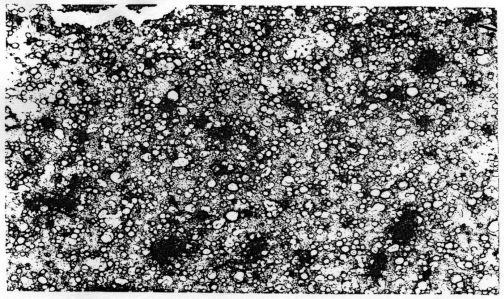
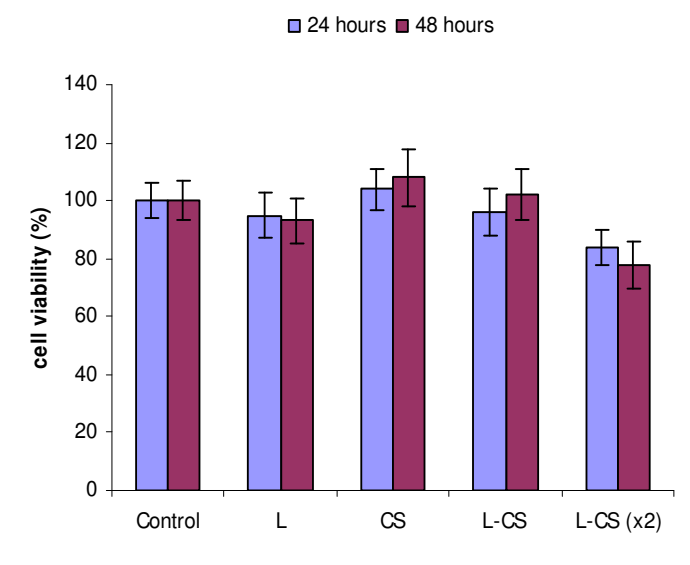


image of SUV (x25000) by negative staining electron microscopy technique

Trif et al., ROMJIST, 10, 85, 2007

Patent OSIM 2007 00838

Biocompatibility tests: 1. HFs viability in the presence of liposome/CS

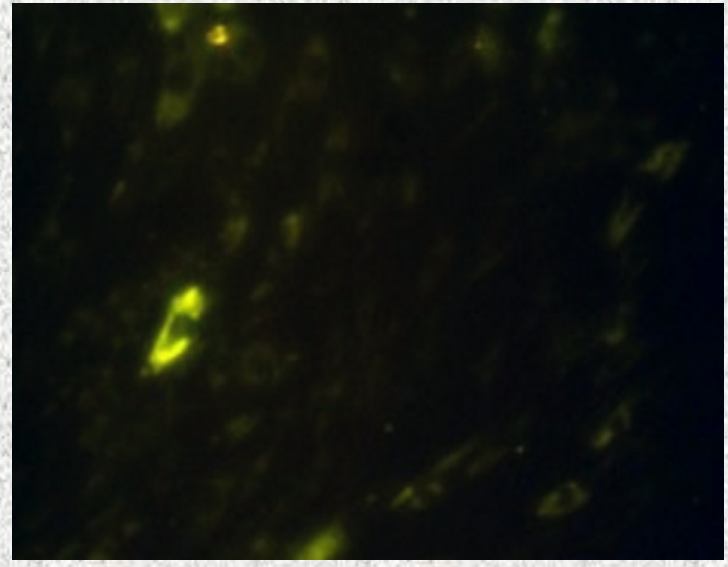


Formulation code	Lipid conc. (µM)	CS Conc. (µg/ml)
L	200	-
CS	-	250
L-CS	**200**	**250**
L-CS(x2)	200	500

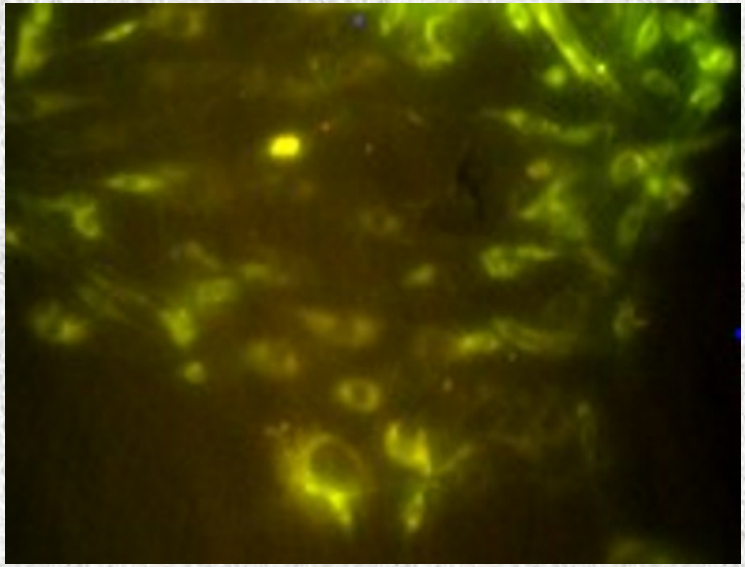
Cell viability test (MTT assay) showed a good viability for fibroblasts incubated with empty liposomes, CS and all liposomal formulations of CS.

Trif et al., Micron 39,1042 ,2008

Liposome ability for cytoplasmic delivery of CS in HFs



Human dermal fibroblasts (HDF) incubated for 24 hours with free compound (CS) (immunofluorescence reaction using Ab anti-CS

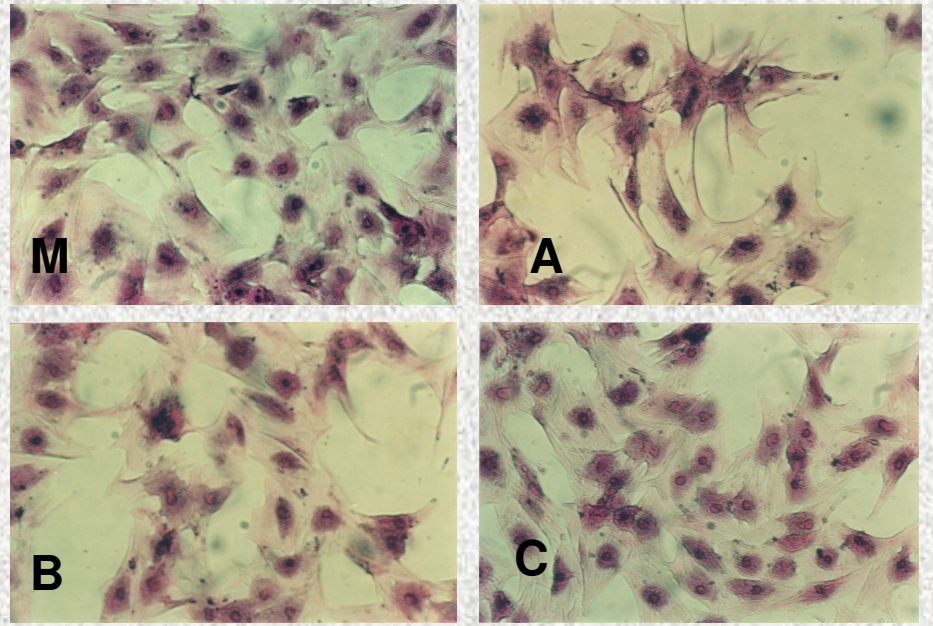


Human dermal fibroblasts (HDF) incubated for 24 hours with liposome containing a hydrophylic compound (CS). Liposomes attached to cell membrane were washed very well. Only intracellular imunofluorescence is observed.

Anti-inflammatory activity of liposome -CS in HCs treated with IL β1

In vitro model of inflammation induced with _IL β1 (100ng/ml)_

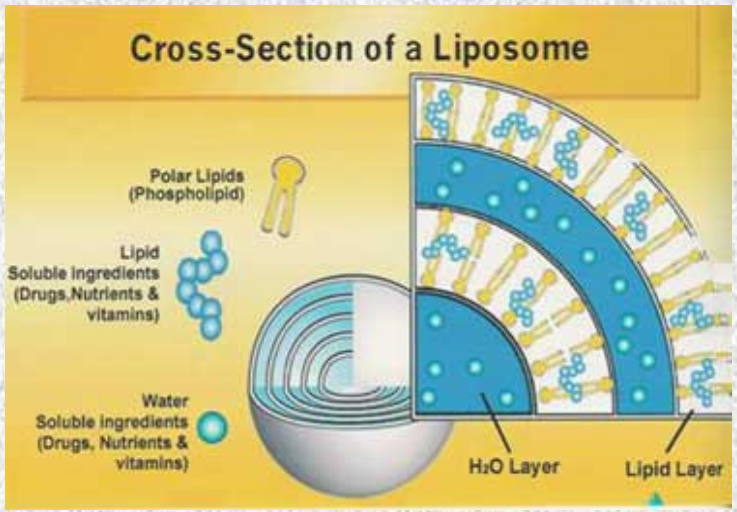
-Control (M) - normal cells; human chondrocytes)
-IL β1 (A) - morphological changes (multiple extensions and stelate shape; also fusiform aspect)
-CS (B) (normal morphologic aspect)
-L-CS (C) (normal morphologic aspect)



Liposomal CS are more efficient in reducing the inflammation induced in HC

Anti-inflammatory activity of liposome systems containing lipophilic drug

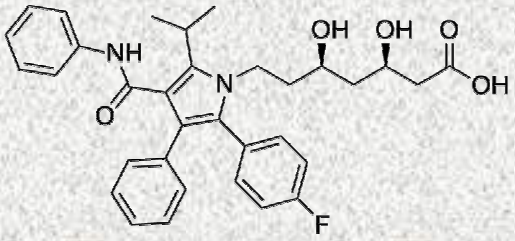
- As lipophilic drug we have proposed a statine known to inhibit synthesis of cholesterol
- **In addition, it was found to exert anti-inflammatory and immunomodulatory actions.**
- We therefore studied the anti-inflammatory effect of liposome based statins in HFs from oral tissue since periodontitis represent a chronic, progressive inflammatory disease
- Detection the level of proinflammatory cytokines that mediates periodontal tissue destruction (TNF α, IL 6, IL 8) in cell culture.



multilamellar vesicle (MLV)
(available on-line from Encapsula NanoSciences)

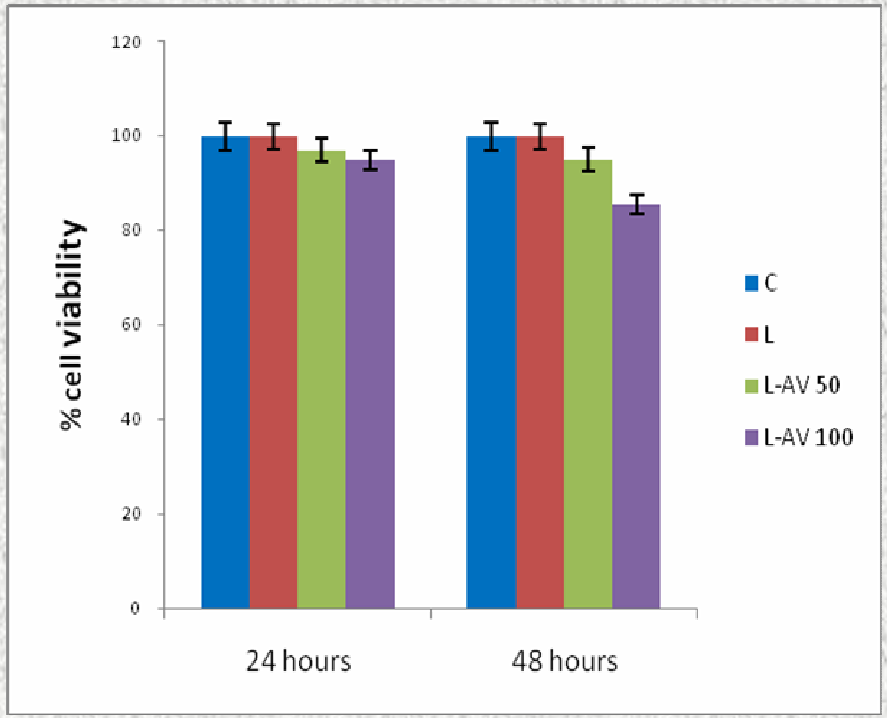
Statins therapeutic activities

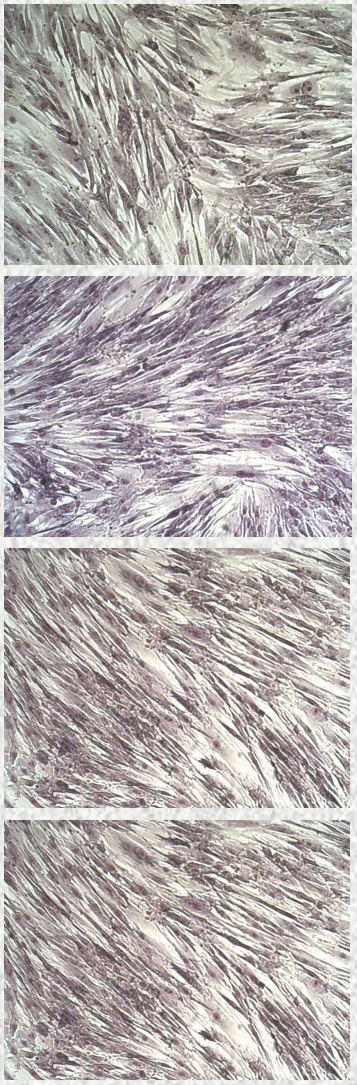
- Improve endothelial function
- Modulate inflammatory responses
- Maintain plaque stability
- Prevent thrombus formation



Lipophilic drug- statine

HFs proliferation and morphology in the presence of liposome-AV

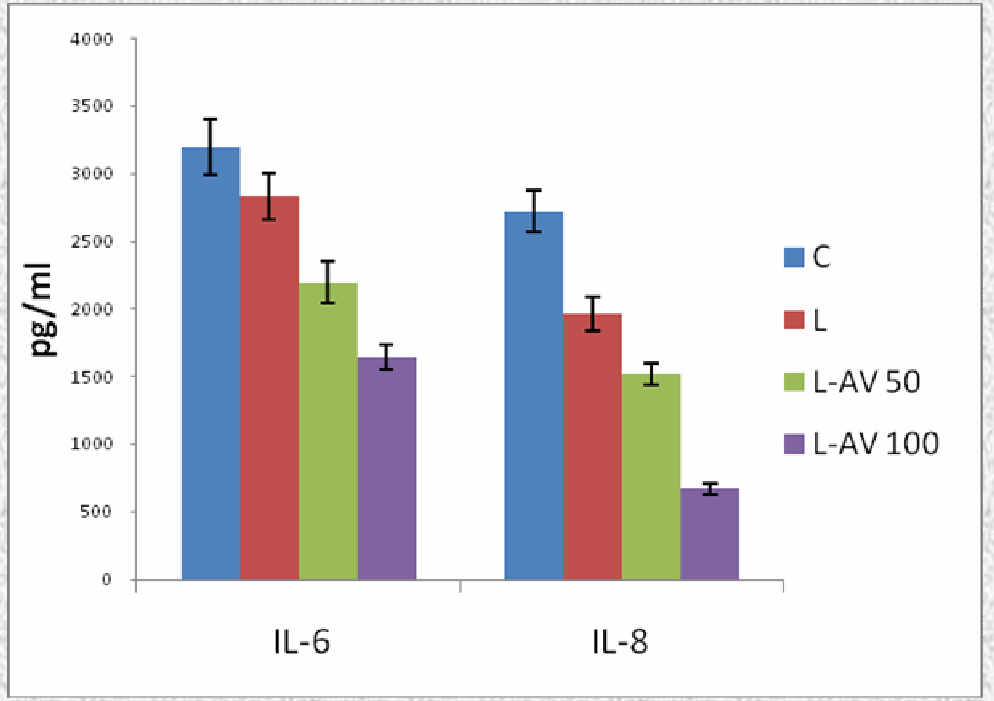




HFs retain their normal phenotype and proliferate in the same way as control cells

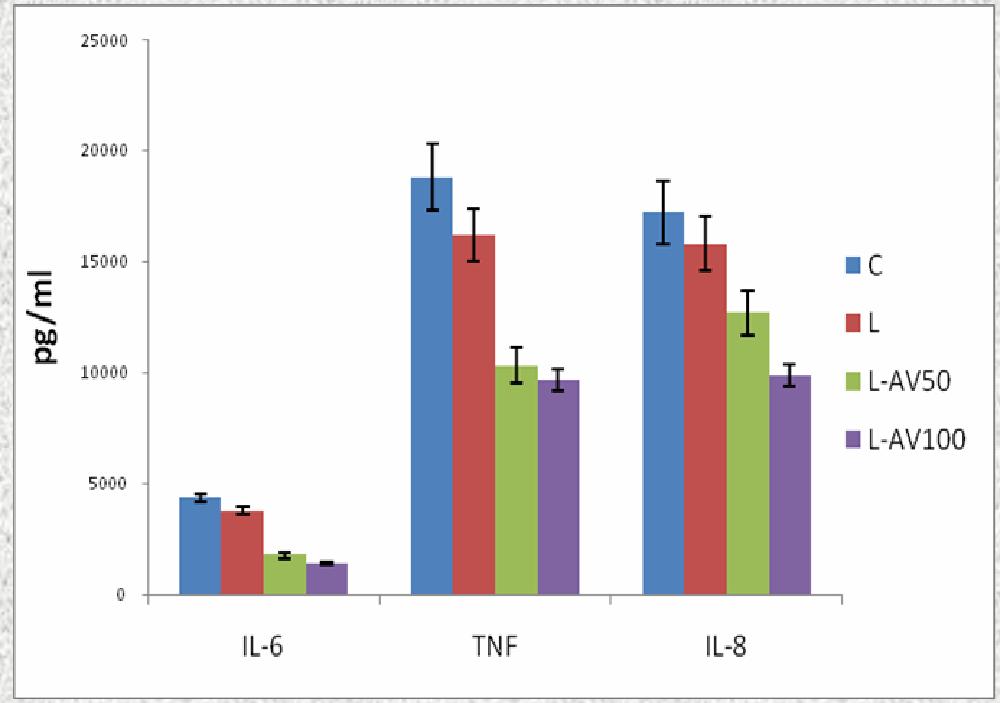
Effect of liposome based statins in HFs from oral tissue

- The level of proinflammatory cytokines that mediates periodontal tissue destruction (IL 6, IL 8) was measured in HFs (*IL β1* induced inflammation)



The anti-inflammatory activity of liposome/statine in THP 1 cells stimulated with LPS

Reduce the level of pro-inflammatory cytokines (TNFα, IL6, IL8)



THP-1 cells

↓

LPS ± Lipo/Lipo-AV

↓

18 h

↓

Cell supernatant

↓

cytokine secretion

TNFα, IL-6, IL-8

CONCLUSIONS

- Biocompatibility of free and liposome-entrapped anti-inflammatory molecules with cell culture HFs and HCs incubated with retain their normal phenotype and proliferate in the same way as control cells.

- Liposome system containing hydrophilic bio-active molecules in the aqueous interior and lipophilic drugs inserted in the lipid bilayer reduce the level of proinflammatory cytokines in different models of inflammation

- **Our findings demonstrated liposome efficiency in increasing the protective and anti-inflammatory effect of pharmacological active molecules.**

Future Research Focus

- Cell Culture Studies
  Cellular and Molecular Studies
- Animal Studies and clinical evaluation

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