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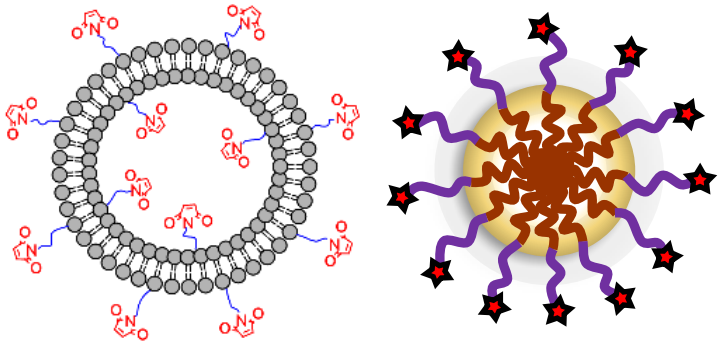
MALEIMIDE-FUNCTIONALISED LIPOSOMES & NANOPARTICLES AS MUCOADHESIVE VEHICLES FOR DRUG DELIVERY TO URINARY BLADDER



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KAHAK Scientific and Technical Society,
Almaty
20th September 2018

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Bladder cancer has the 9th highest incidence rate worldwide, with a greater prevalence among men than women.

In the UK:

- **10,100 new cases of bladder cancer in 2014, that's 28 cases diagnosed every day.**
- **BC is the 10th most common cancer (2014).**
- **In males, BC is the eight most common cancer and 14th in females**

https://www.iarc.fr

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In Kazakhstan:

- **3,300 registered cases in the beginning of 2013**
- **More than 600 new cases of bladder cancer each year, that's 2 cases diagnosed every day.**
- **BC is the 10th most common cancer (2013).**
- **In males, BC is the ten most common cancer and 13th in females**

Признаки и симптомы рака мочевого пузыря включают:

- **Кровь в моче**
- **Частое мочеиспускание**
- **Боль во время мочеиспускания**
- **Боль в пояснице**

Факторы риска развития рака мочевого пузыря включают:

- **Использование табака, особенно курение сигарет**
- **Наследственный фактор, или предрасположенность к болезни**
- **Краски, красители, металлы или нефтепродукты на рабочем месте**
- **чрезмерное потребление алколголя**
- **Питьевая вода из скважины с высоким содержанием мышьяка**
- **Питьевая вода, обработанная хлором**

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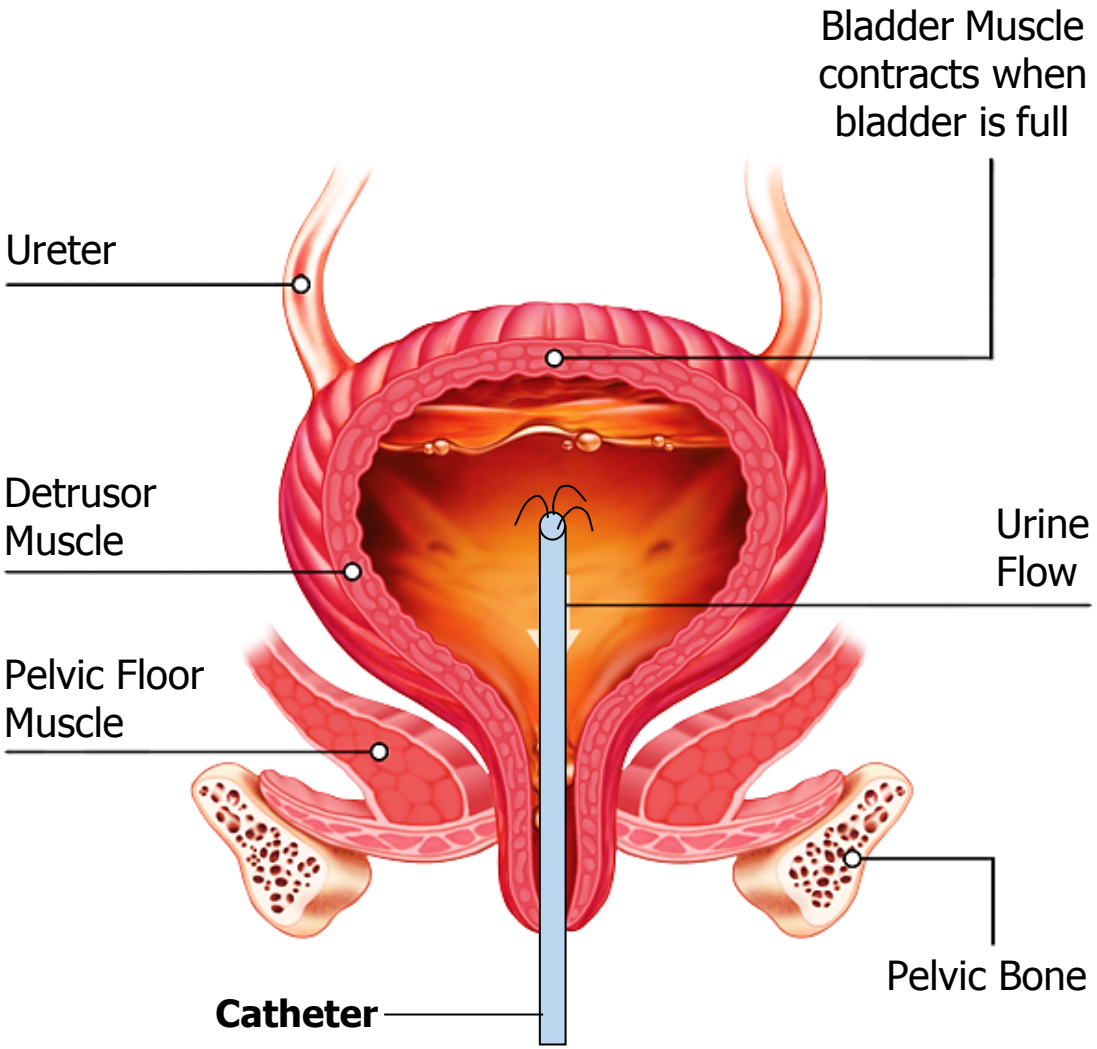
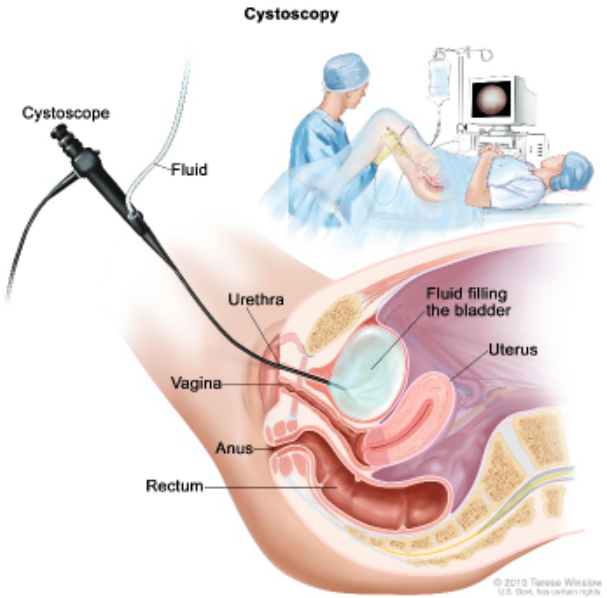
Urinary bladder: intravesical delivery



- Normal capacity: 400–600 mL;
- 150–300 mL triggers the urge to urinate;
- Urinary bladder wall is highly impermeable



http://peeingproblem.ca/en/what_is_oab/how_normal_bladder_works/

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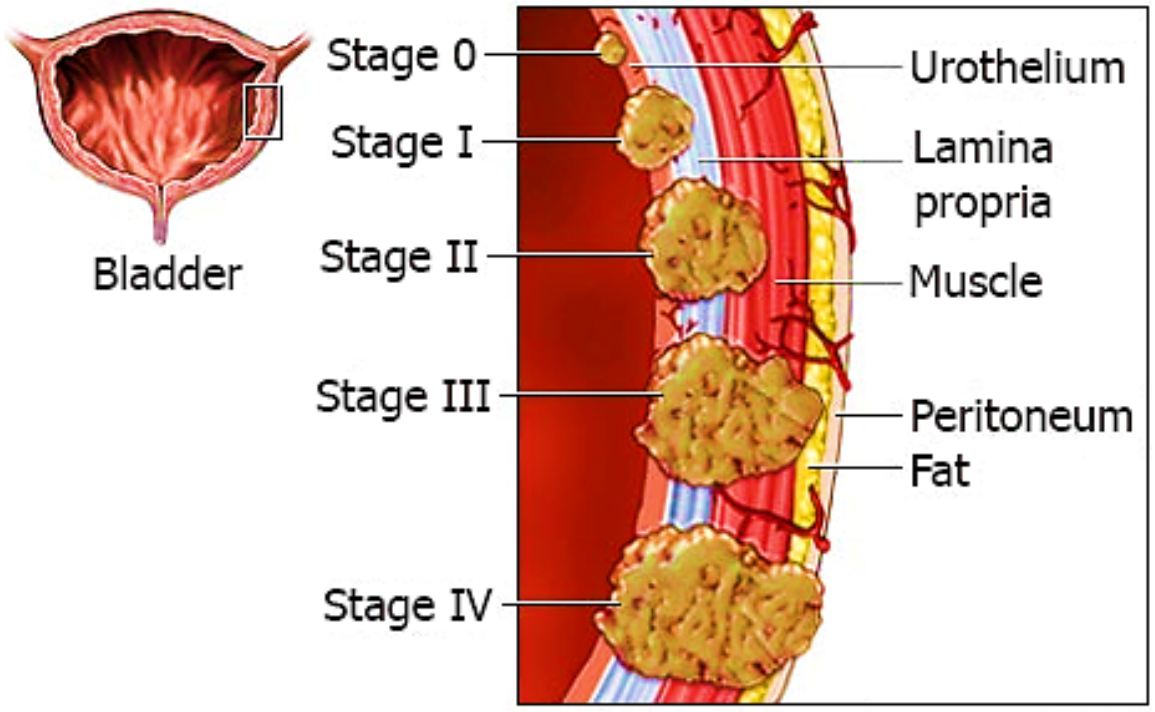
Bladder cancer

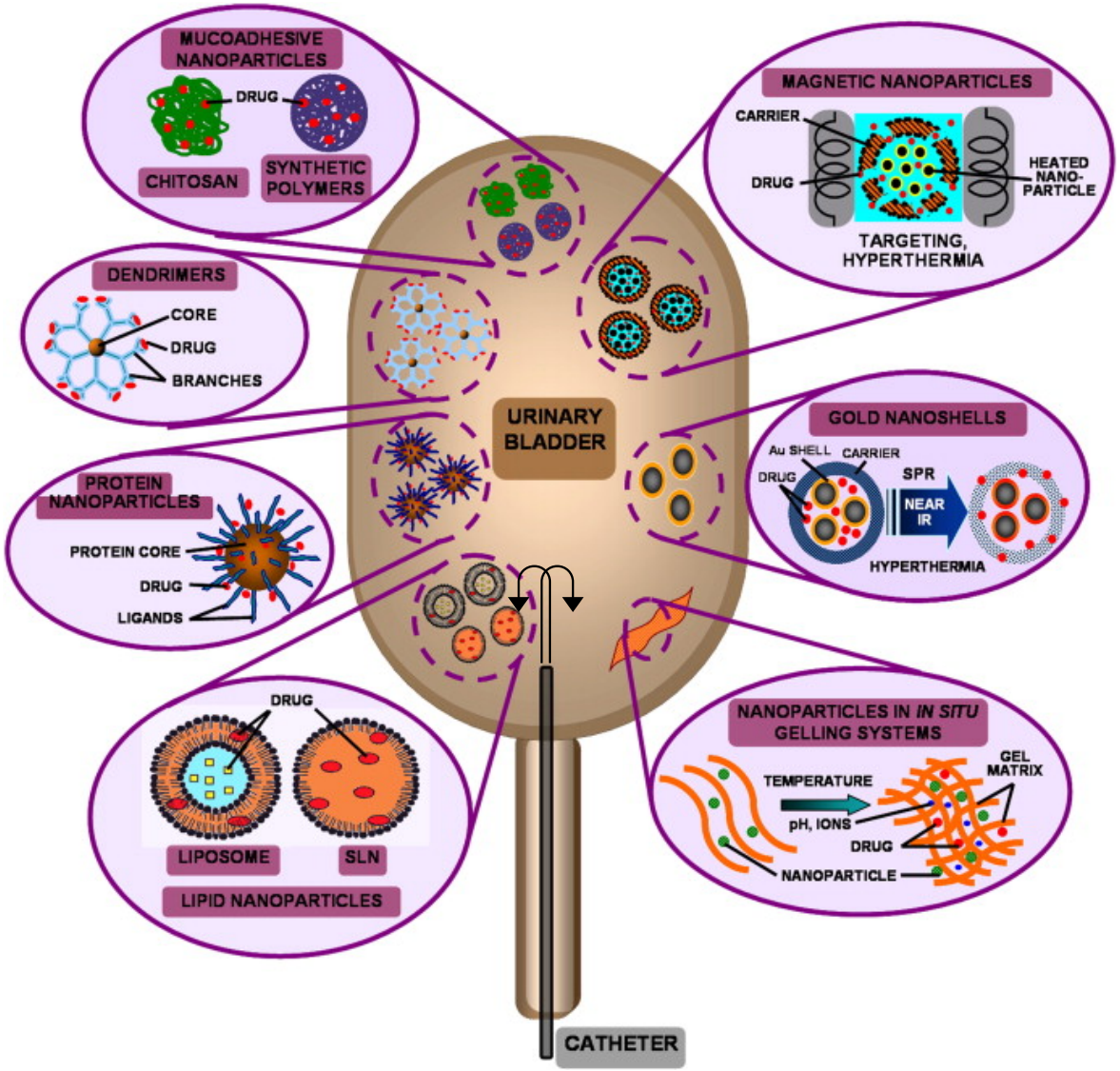


Intravesical therapy is used only for non-invasive (stage 0) or minimally invasive (stage I) bladder cancers.

Intravesical immunotherapy: Bacillus Calmette-Guerin (BCG)

Intravesical chemotherapy: Mitomycin, valrubicin, doxorubicin, and gemcitabine

http://www.urologycenter.com/conditions.cfm


MUCOADHESIVE NANOPARTICLES
DRUG
CHITOSAN
SYNTHETIC POLYMERS
MAGNETIC NANOPARTICLES
CARRIER
DRUG
HEATED NANO-PARTICLE
TARGETING, HYPERTHERMIA
DENDRIMERS
CORE
DRUG
BRANCHES
URINARY BLADDER
GOLD NANOSHELLS
Au SHELL
CARRIER
DRUG
SPR
NEAR IR
HYPERTHERMIA
PROTEIN NANOPARTICLES
PROTEIN CORE
DRUG
LIGANDS
DRUG
LIPOSOME
SLN
LIPID NANOPARTICLES
NANOPARTICLES IN IN SITU GELLING SYSTEMS
TEMPERATURE
pH, IONS
GEL MATRIX
DRUG
NANOPARTICLE
CATHETER

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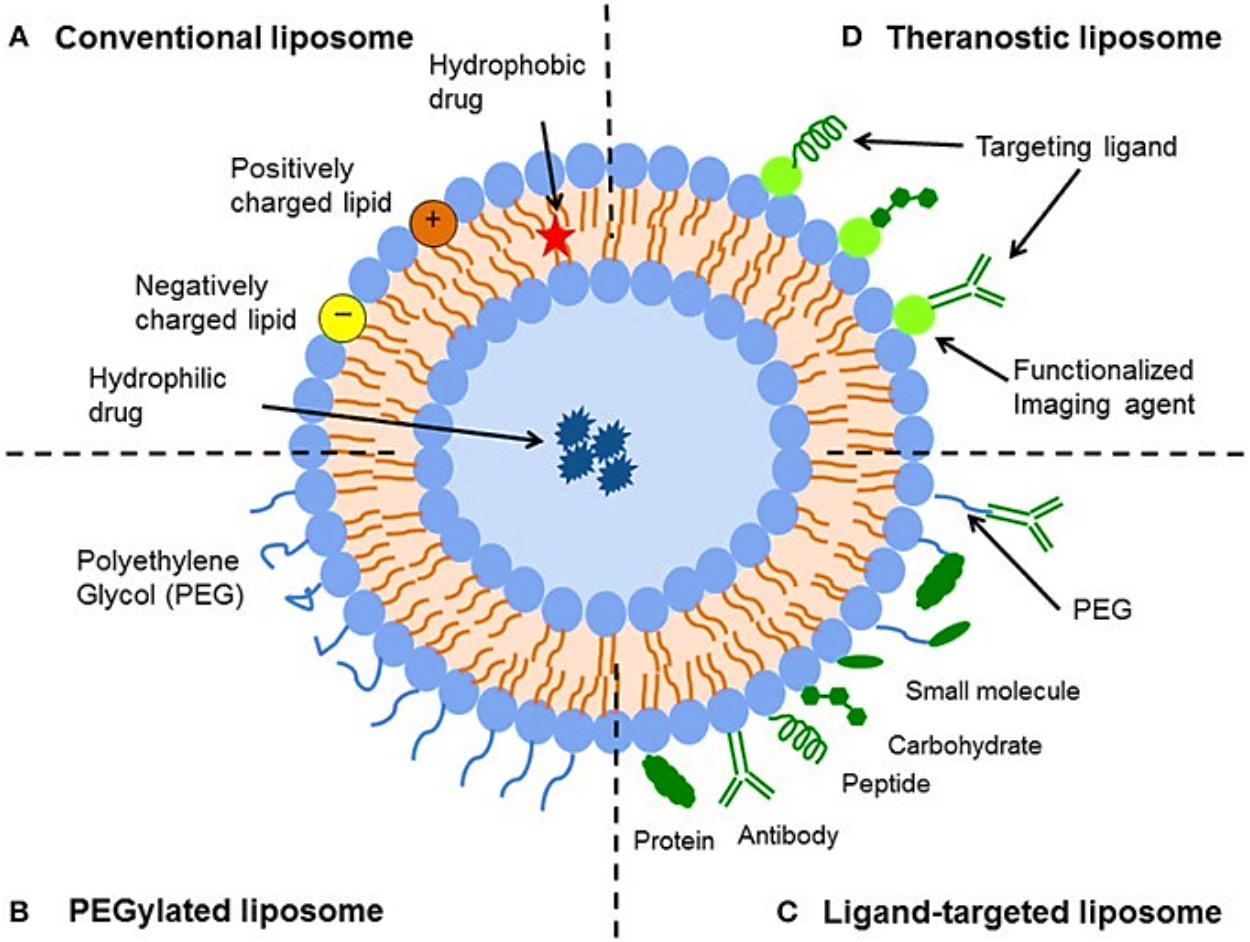
Types of liposomes



- Non-toxicity, biocompatible, and completely biodegradable
- Increasing drug efficacy
- Site avoidance effect
- Increasing stability via encapsulation process
- Reducing the toxicity of encapsulated drugs

Frontiers in Pharmacology, **2015,** *6,* 1–13

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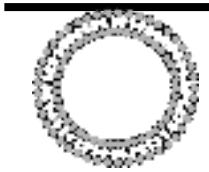
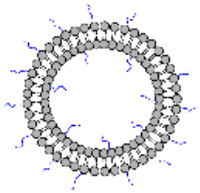
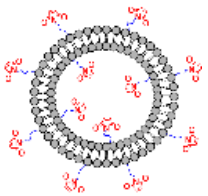
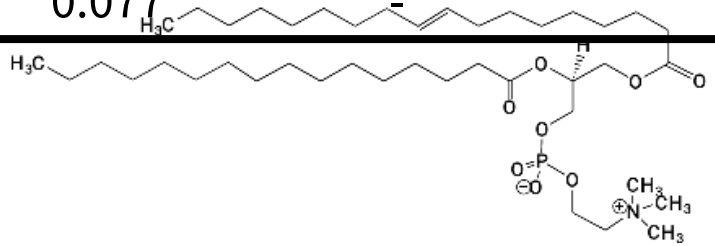
THE COMPOSITION OF LIPOSOMAL FORMULATIONS

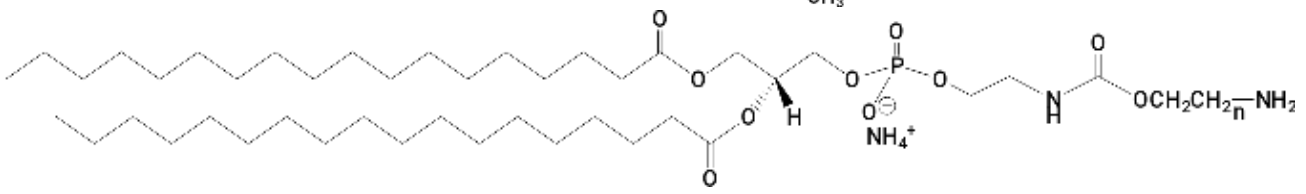
Liposome formulations	PC (%)	Chol (%)	PEG_{2000}-DSPE (%)	PEG_{2000}-DSPE-Mal (%)	NaFlu (%)
Conventional	0.773	0.077	-	-	0.2
PEGylated	0.773	0.077	0.075	-	0.2
PEG-Mal	0.773	0.077	-	0.075	0.2

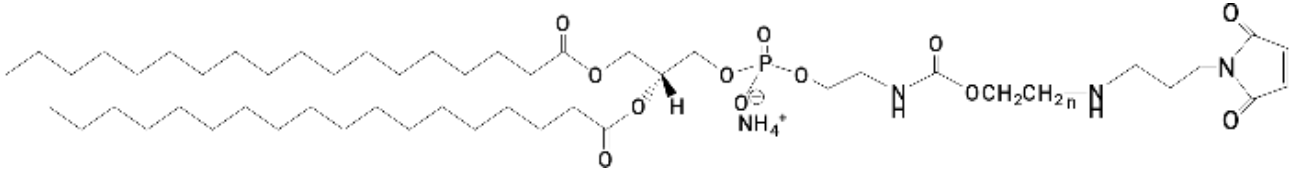












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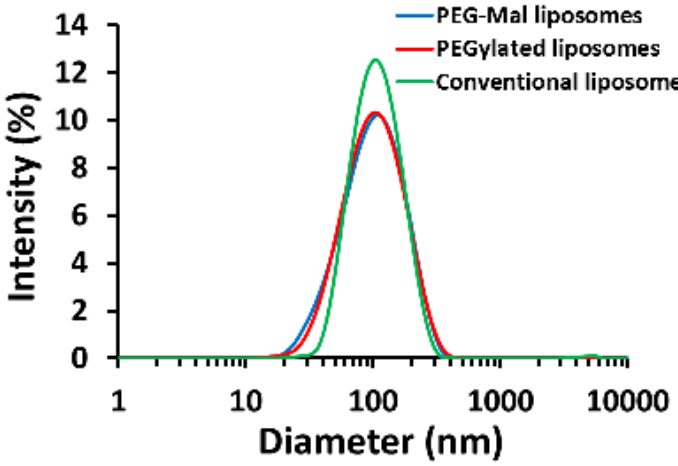
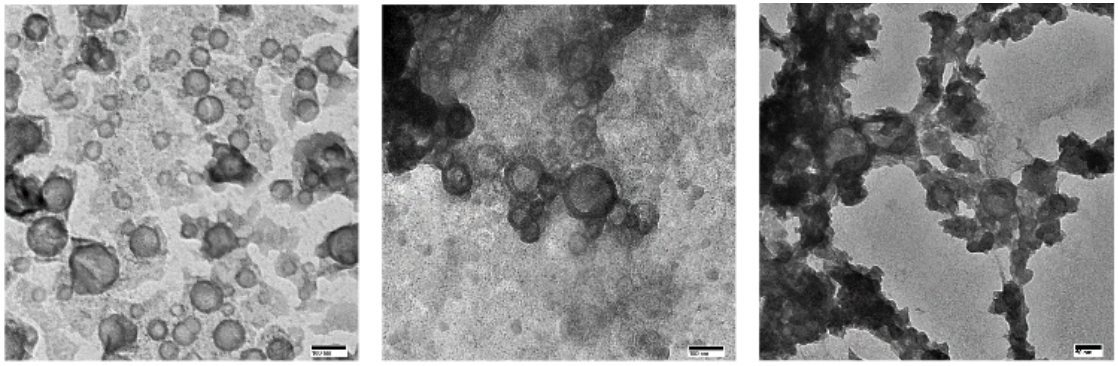
PHYSICOCHEMICAL CHARACTERISTICS

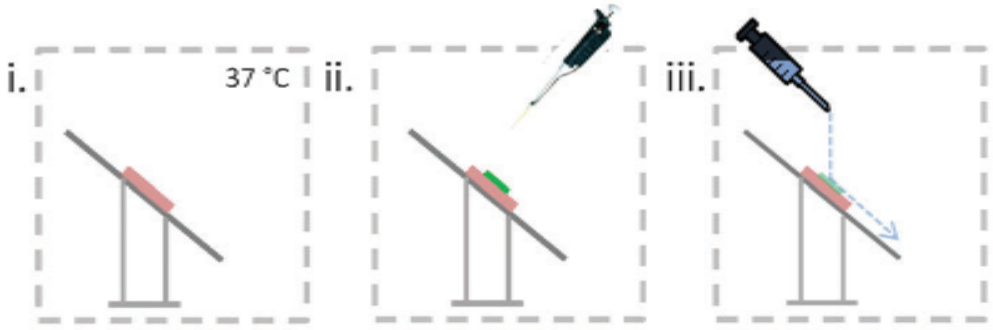
Liposome formulations	Mean diameter (nm)	PDI	Zeta potential (mV)	%EE	%LC
Conventional	97 ± 1	0.145	-53 ± 1	53 ± 6	12 ± 1
PEGylated	85 ± 1	0.217	-32 ± 2	27 ± 2	6 ± 1
PEG-Mal	86 ± 1	0.224	-37 ± 1	25 ± 2	5 ± 1





Scale bars are 100 nm for CL and PEG, and 50 nm for PEG-Mal

Application of mucoadhesive onto a bladder mucosa



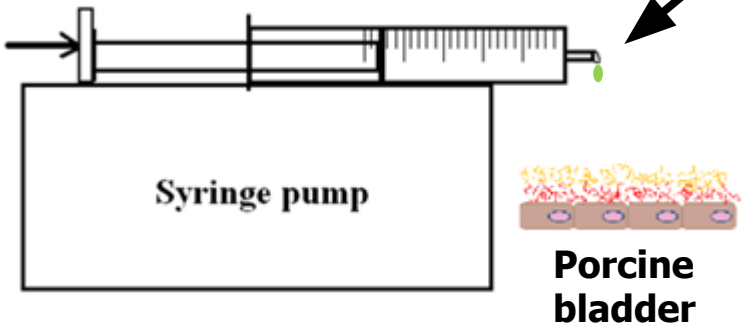
Chem. Commun., **2015**, *51*, 14447-14450

Fluorescence microscopy

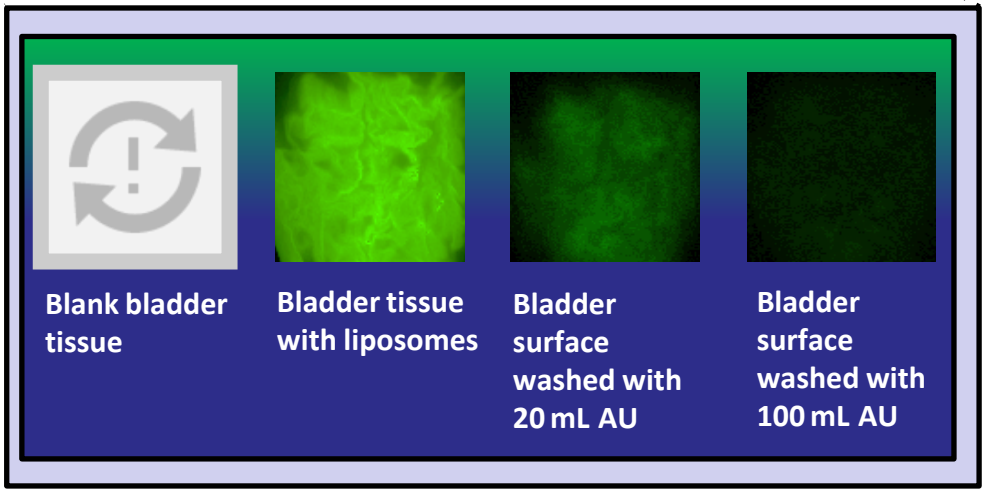
ImageJ

Image Processing & Analysis in Java

Artificial urine



Porcine bladder



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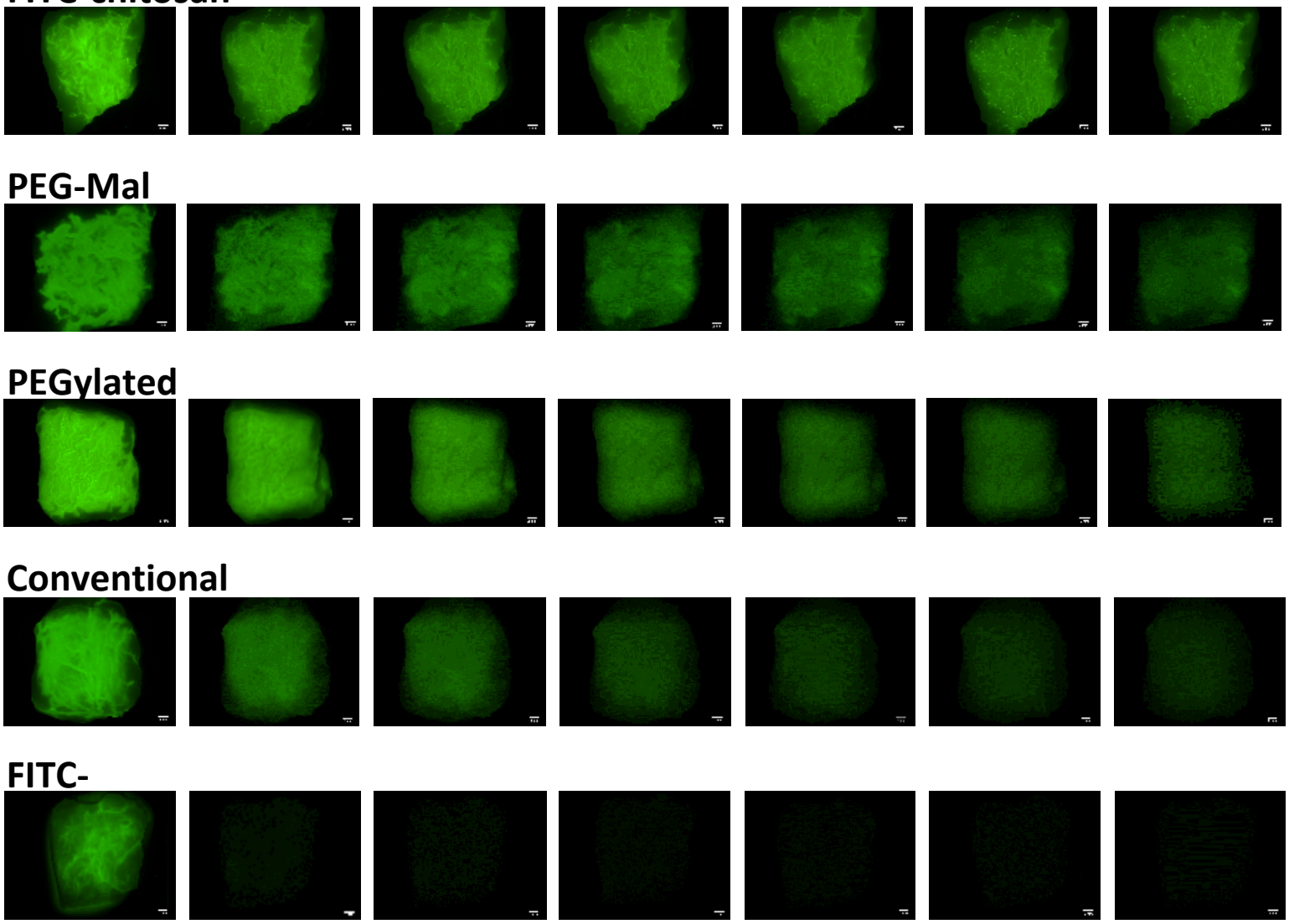
Exemplary fluorescent images of the retention of formulations on porcine urinary bladder mucosa



Scale bars are 2 mm

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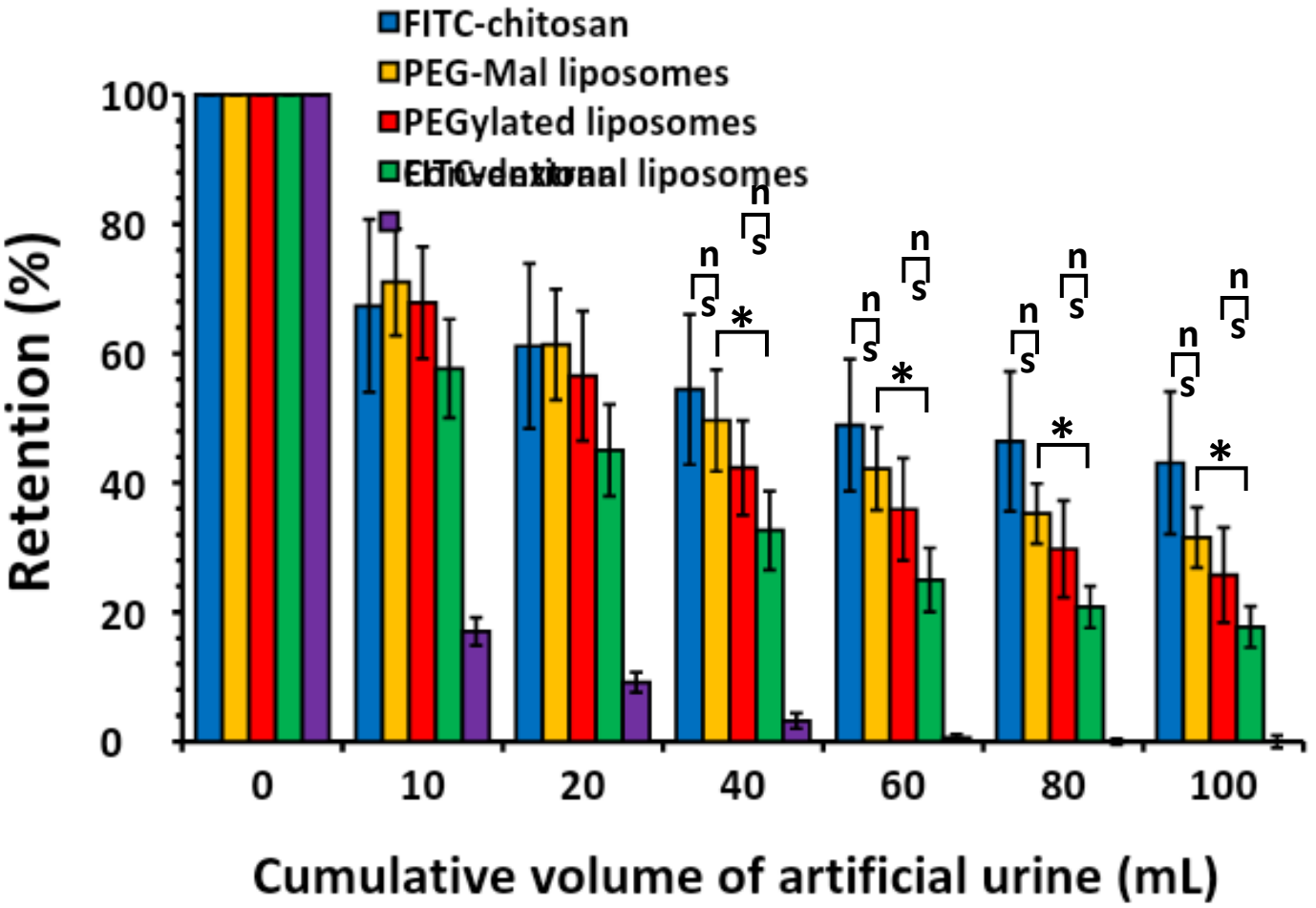
Retention of formulations on porcine urinary bladder



Statistically significant differences are given as: * = p < 0.05; ns – no significance

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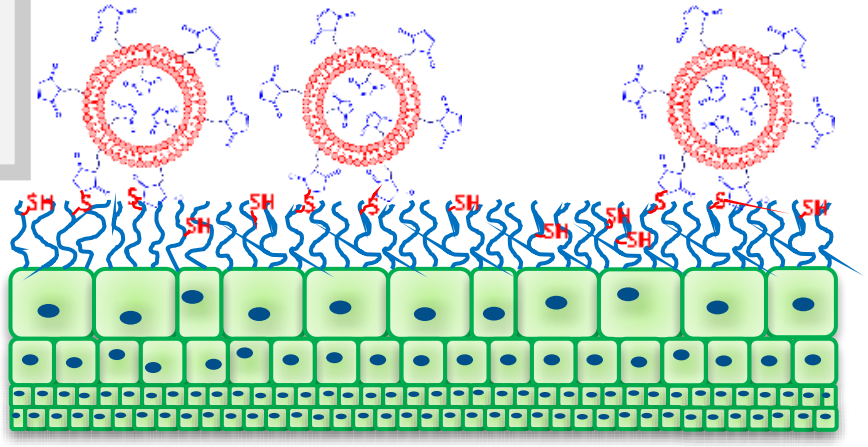
WASH OUT$_{50}$ PROFILES

Formulations	WO_{50}, mL
Conventional	15
PEGylated	24
PEG-Mal	48
FITC-chitosan	91
FITC-dextran	5



Proposed mechanism of bonding between maleimide-functionalised liposomes and mucosal surfaces

Wash Out$_{50}$ (WO_{50}) values are defined as the volume of liquid necessary to remove 50% of a mucoadhesive material from a substrate

Int. J Pharm., **2016**, *512*, 32-38

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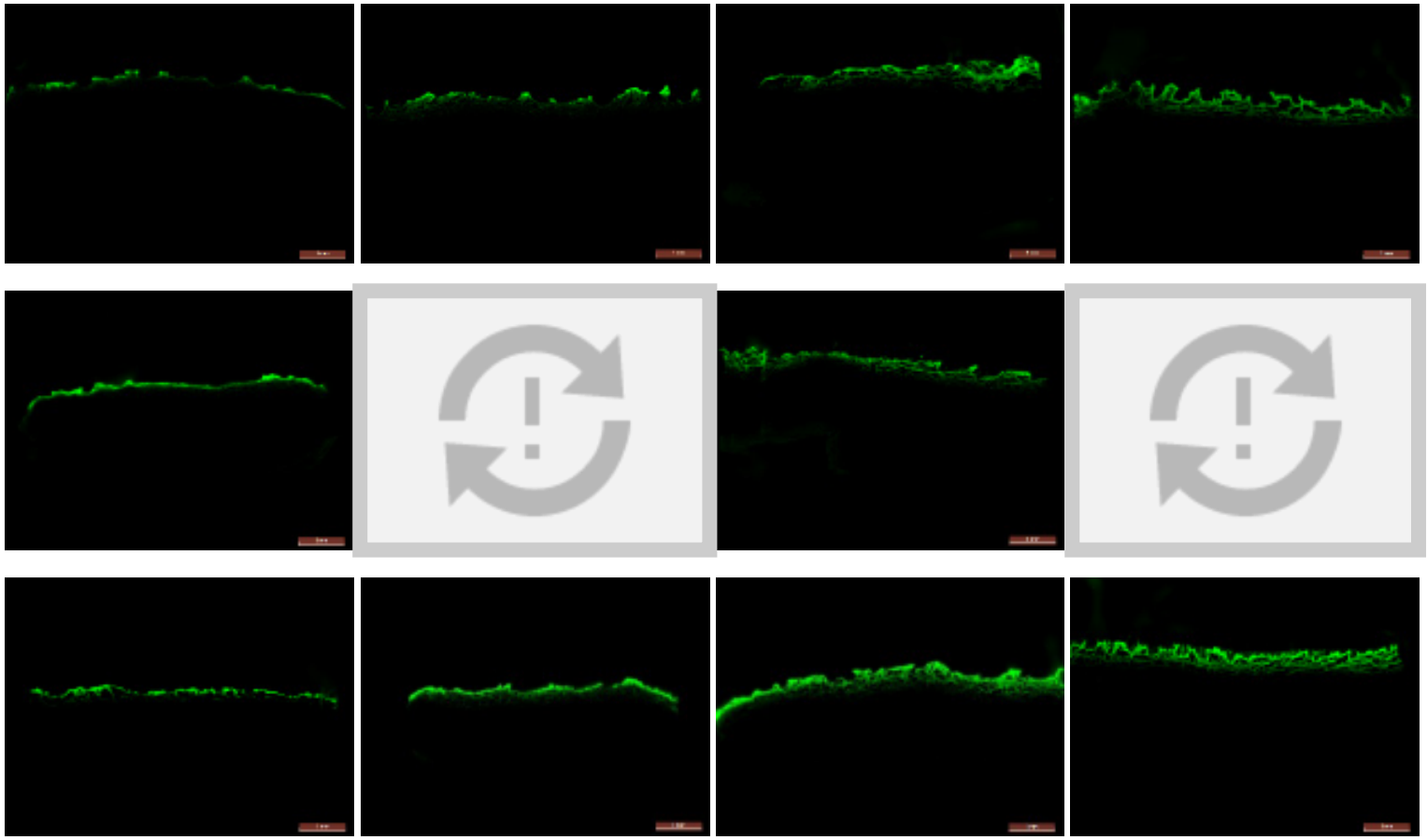
Penetration into bladder mucosa

Exemplary fluorescence microscopy images:



Scale bars are 1 mm

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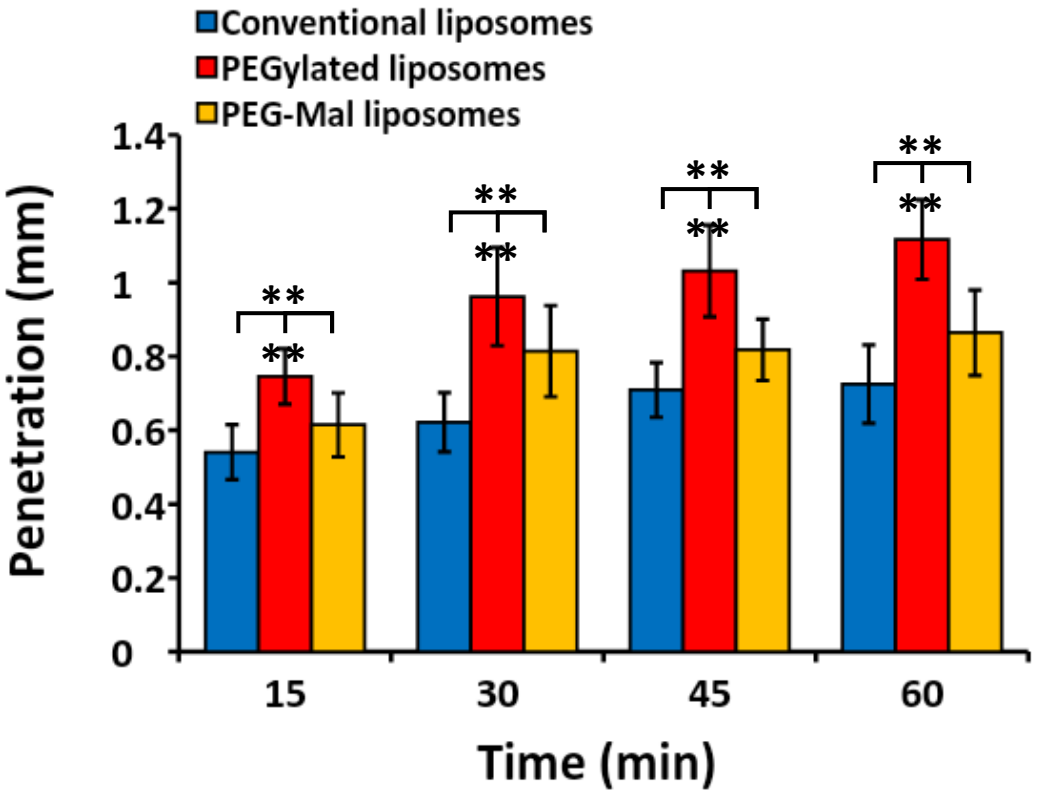
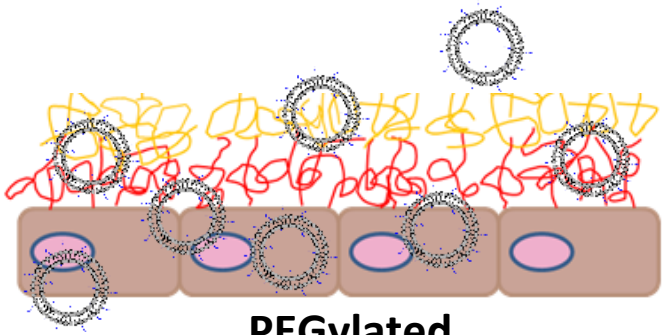
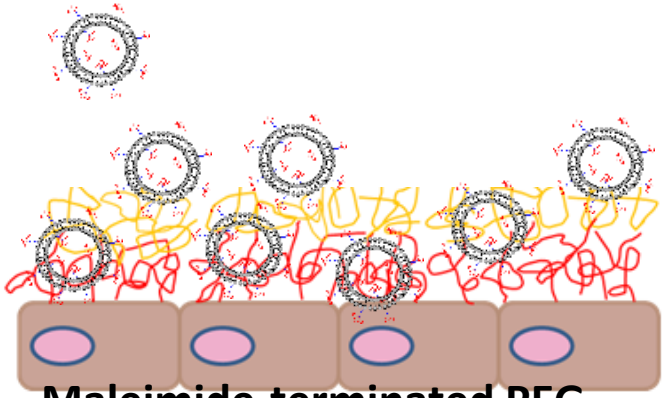
Penetration into bladder mucosa





PEGylated liposomes



Maleimide-terminated PEG liposomes

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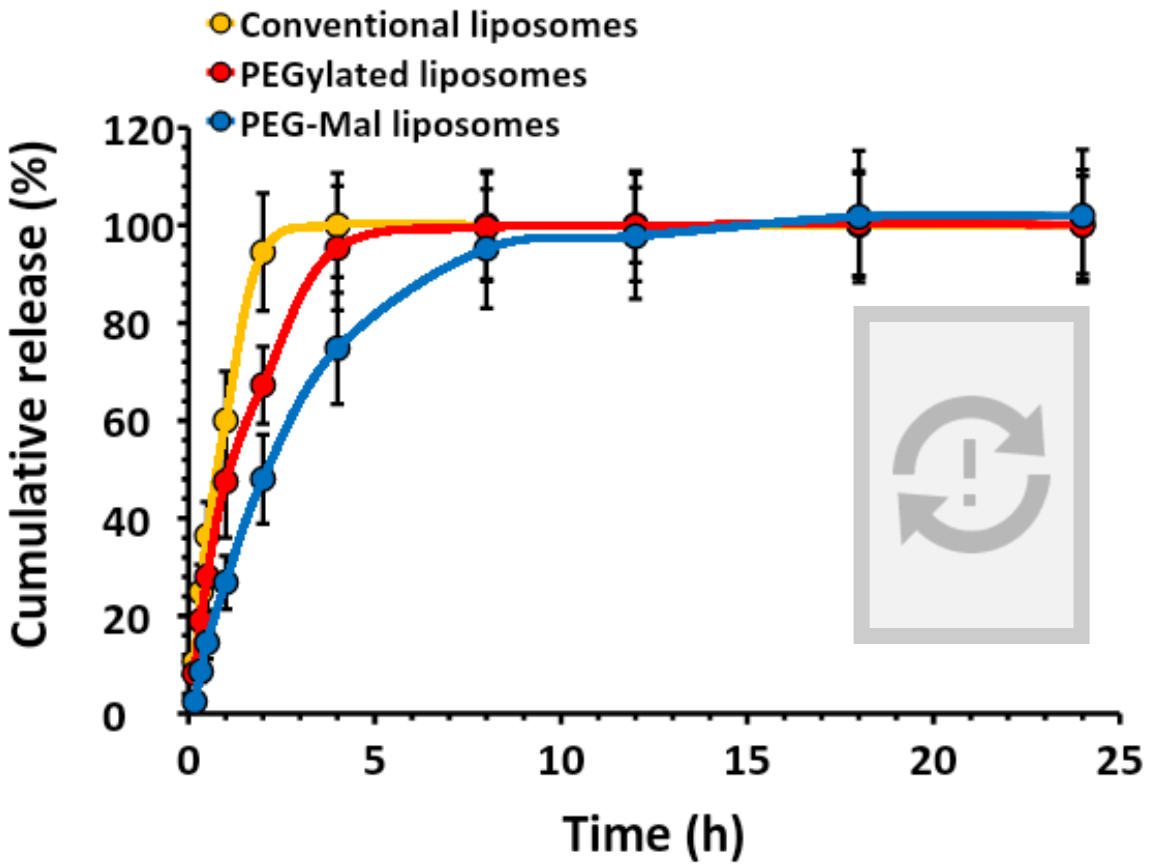
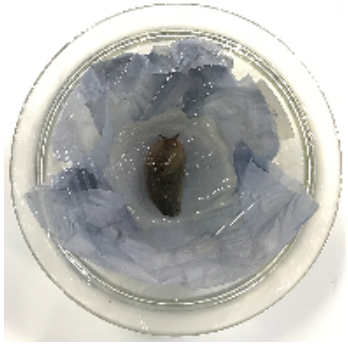
In vitro release profile



Toxicity – Slug mucosal irritation test

METHOD:

1



Kept in glass beakers lined with paper towels soaked with 20 mL of PBS at RT for 48 h

Slugs sourced from UoR Harris Garden, Reading

2

weighed before the experiment

filter paper moistened with test materials

Left to contact for 1 h

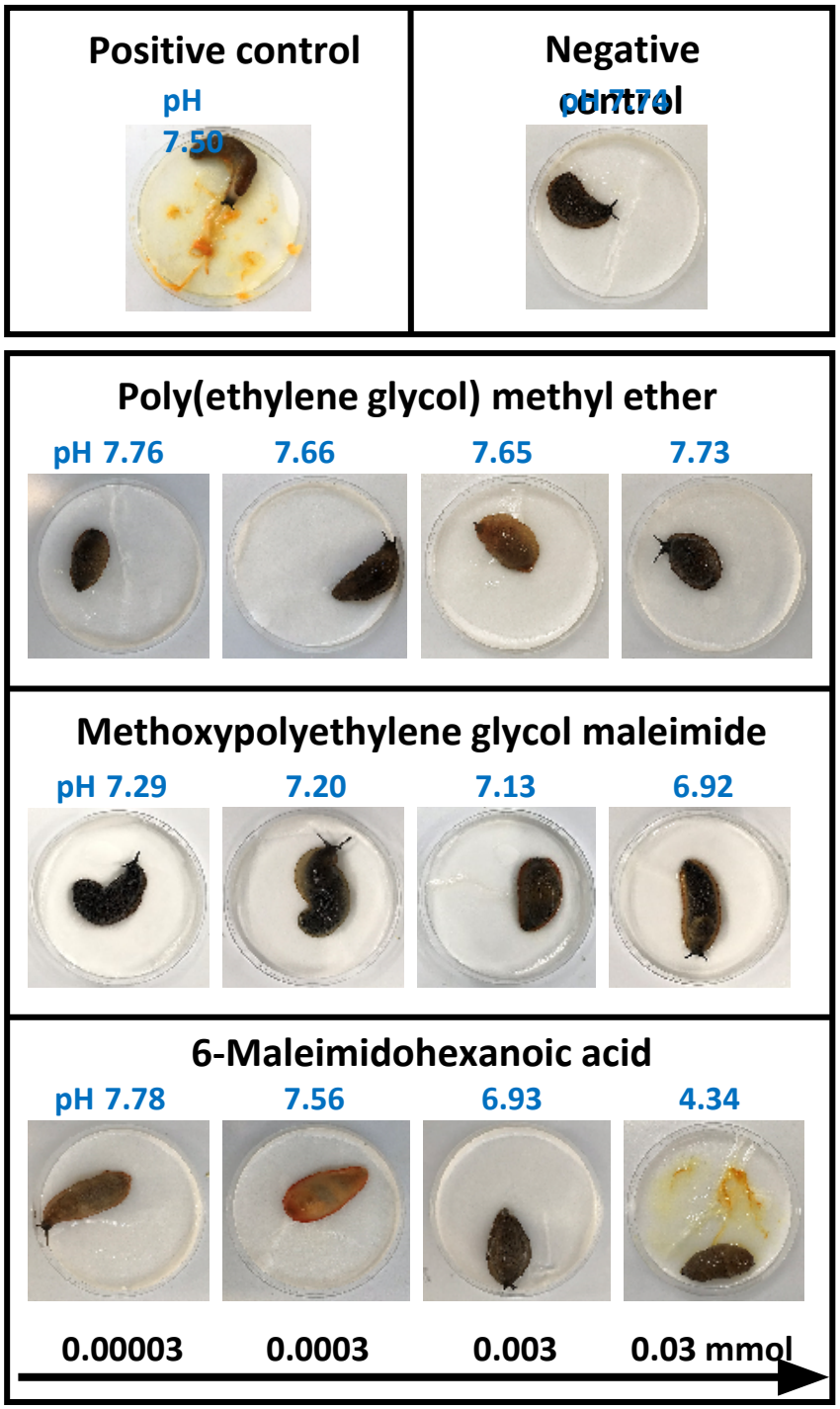
3

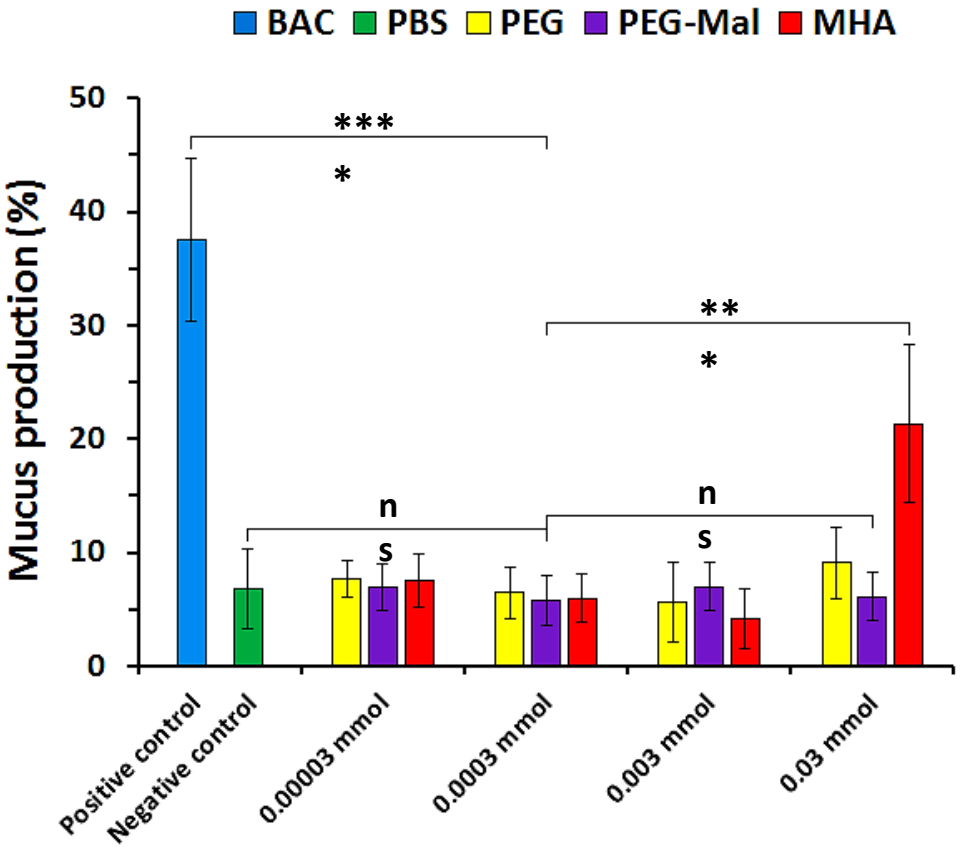
rinsed and gently wiped with PBS

re-weighed

Mucus production:

$$MP\% = \frac{(m_{before} - m_{after})}{m_{before}} \times 100$$



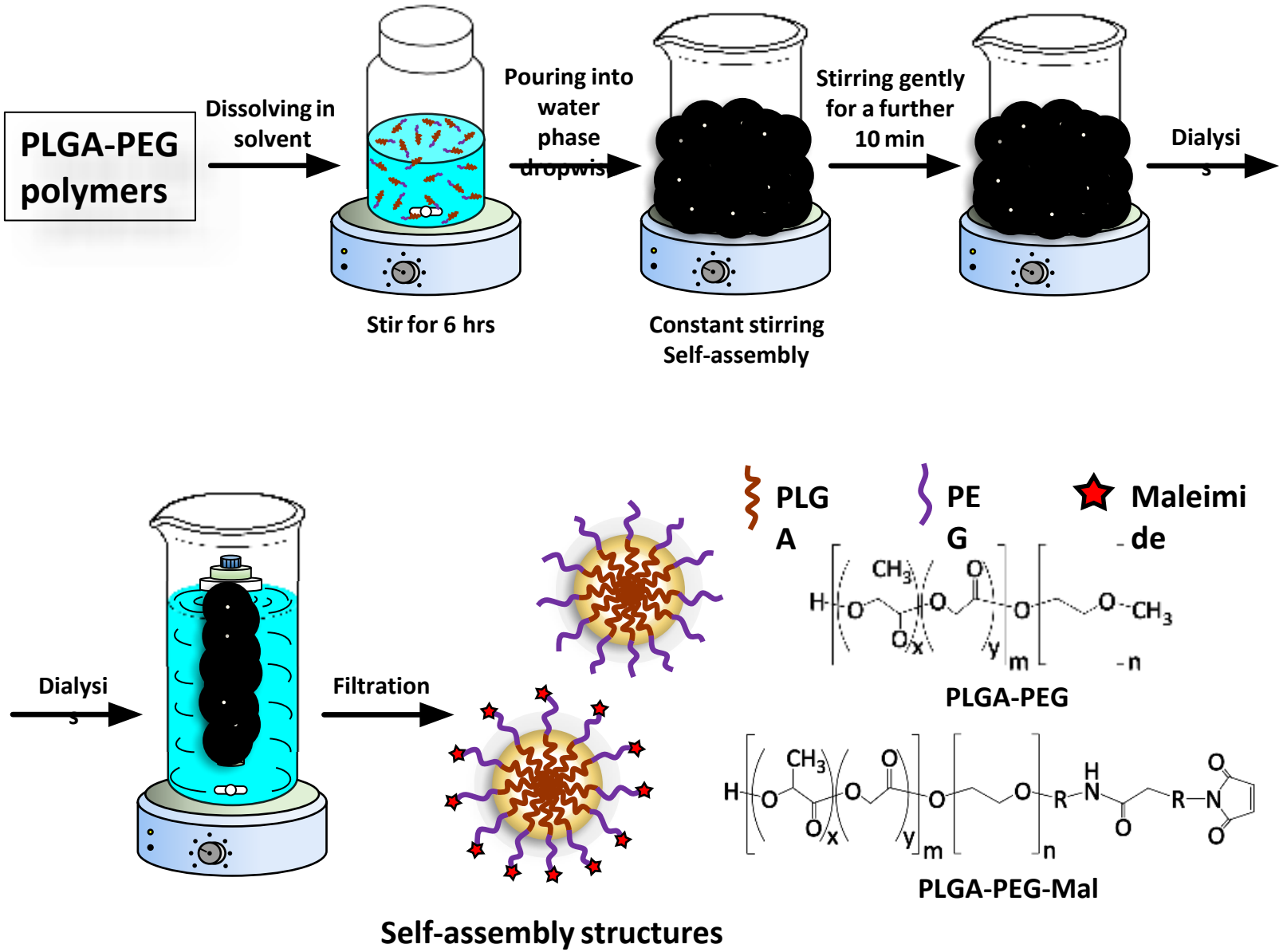


BAC – Benzalkonium chloride
PBS – Phosphate buffered saline
PEG – Poly(ethylene glycol) methyl ether (average Mn 5,000)
PEG-Mal - Methoxypolyethylene glycol maleimide (average Mn 5,000)
MHA – 6-Maleimidohexanoic acid

Results published in

Eur. J Pharm. Sci. **2018**, *111*, 83-90

Schematic diagram showing the preparation of PLGA-PEG NPs



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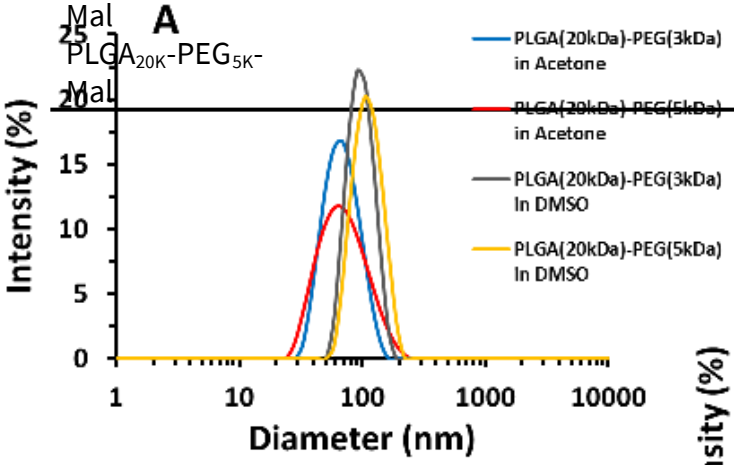
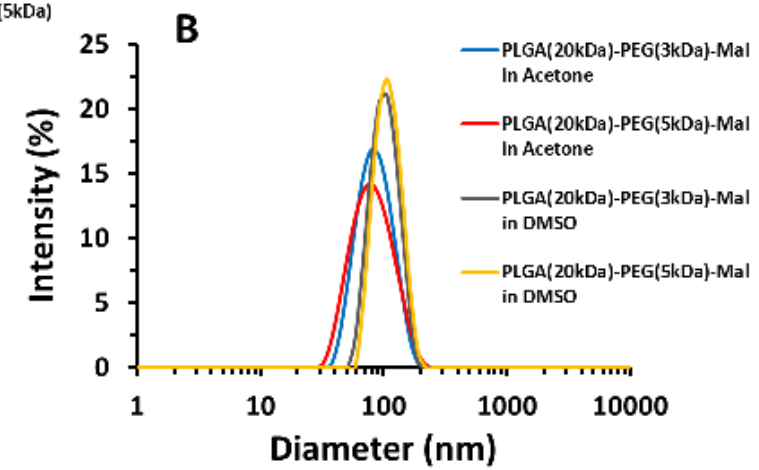
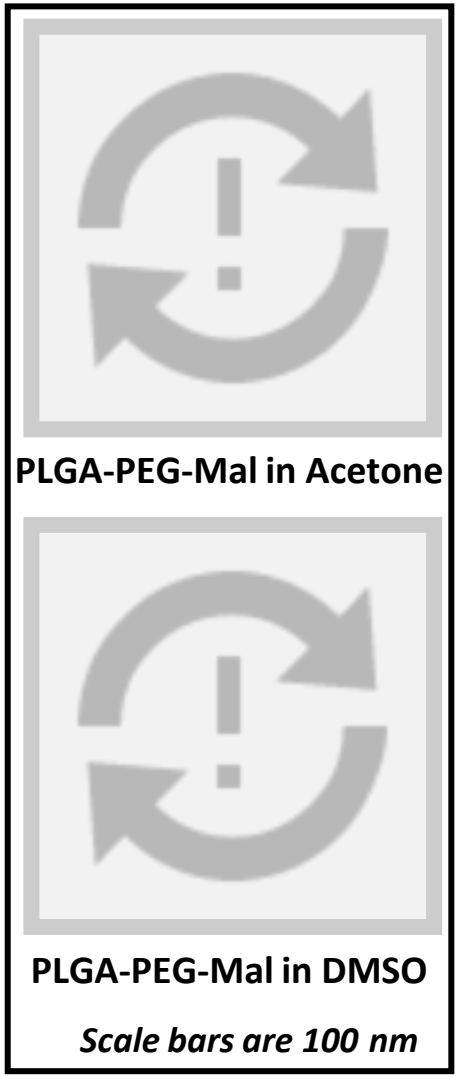
PHYSICOCHEMICAL CHARACTERISTICS

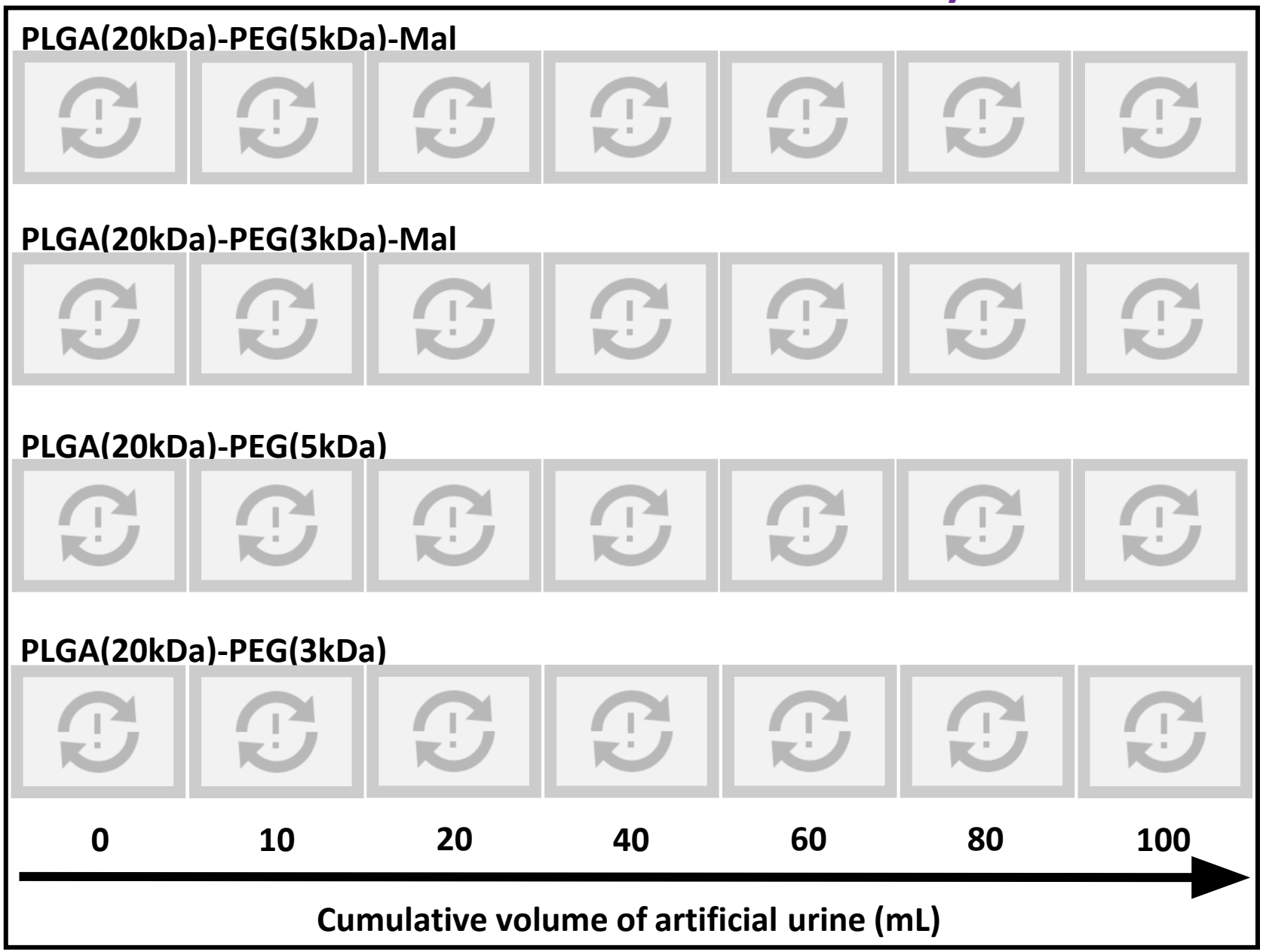
Formulation	Solvent used in NPs preparation	Mean diameter (nm)	PDI	Zeta potential (mV)
$PLGA_{20K}$-PEG_{3K}	Acetone	64 ± 1	0.110	– 8 ± 1
$PLGA_{20K}$-PEG_{5K}		68 ± 1	0.248	– 5 ± 1
$PLGA_{20K}$-PEG_{3K}-Mal		80 ± 1	0.094	– 17 ± 1
$PLGA_{20K}$-PEG_{5K}-Mal		81 ± 1	0.206	– 11 ± 1
$PLGA_{20K}$-PEG_{3K}	DMSO	94 ± 1	0.048	– 21 ± 1
$PLGA_{20K}$-PEG_{5K}		105 ± 1	0.070	– 22 ± 1
$PLGA_{20K}$-PEG_{3K}-Mal		98 ± 1	0.060	– 18 ± 1
$PLGA_{20K}$-PEG_{5K}-Mal		104 ± 1	0.067	– 12 ± 1







Exemplary fluorescent images of the retention of formulations on lamb urinary bladder mucosa



Scale bars are 2 mm

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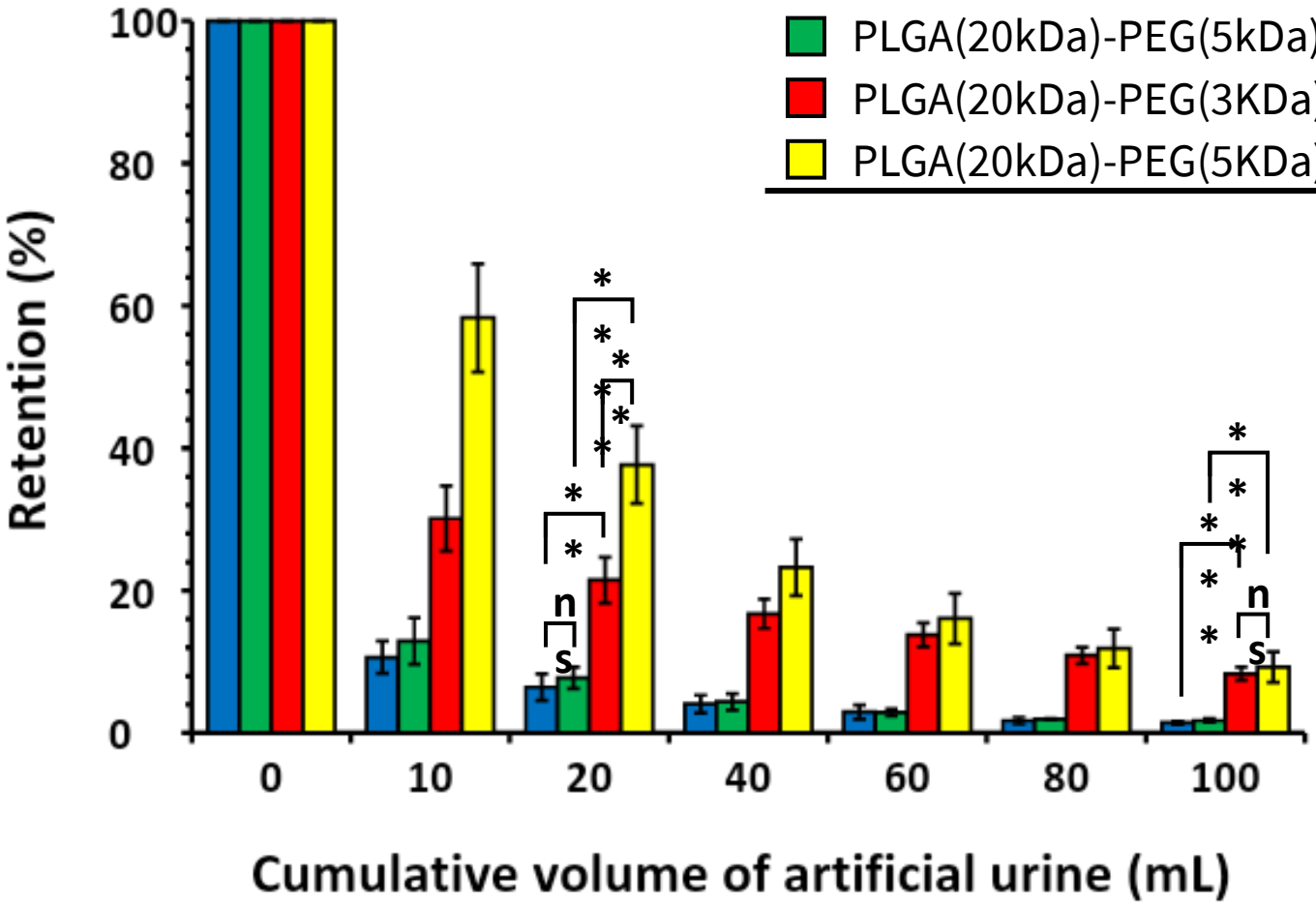
Retention of formulations on lamb urinary bladder

Formulations	WO_{50}, mL
PLGA(20kDa)-PEG(3kDa)	4
PLGA(20kDa)-PEG(5kDa)	5
PLGA(20kDa)-PEG(3KDa)-Mal	7
PLGA(20kDa)-PEG(5KDa)-Mal	13



Statistically significant differences are given as: **** = p < 0.0001; *** = p < 0.001; ** = p < 0.01; ns – no significance.

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CONCLUSION

- Maleimide-functionalised liposomes and PLGA-PEG NPs were prepared;
- Maleimide groups can enhance mucoadhesiveness of particles on urinary bladder;
- These vehicles could be used in intravesical drug delivery.

Пожелания

- **Необходимо проводить ежегодное обследование**
- **Знать свою генетическую предрасположенность**
- **Вести здоровый образ жизни**
- **Исключить курение и алкоголь. И, конечно же, жить положительными эмоциями, то о чем мы думаем, обязательно сбудется.**

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Acknowledgements

Sponsor:

BRITISH COUNCIL

RESEARCHER LINKS

Newton–Al-Farabi Partnership Programme

and all collaborators.

THANK YOU!