New Hyaluronic Derivative as Antibacterial Coating in Orthopaedic Surgery

Matricardi Pietro[a], Cencetti Claudia[a], Meraner Joachim[b], Battista Angela[b], Sacchetta Anna[b], Bellini Davide[b]
[a] Department of Drug Chemistry and Technologies, "Sapienza" University of Rome,, Italy - [b] Novagenit Srl, Mezzolombardo (TN), Italy

Introduction

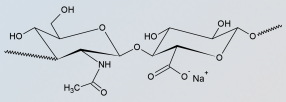
With a share of 38%, orthopaedic and traumatology (O&T) are the worldwide leading markets of implanted biomaterials, involving millions of new patients each year at an increasing trend. Infection related to implanted medical devices is directly related to bacterial capability to establish multilayered, highly structured biofilms on artificial surfaces. Bacterial infections due to implanted biomaterials represent the most devastating complication in O&T, involving millions of patients.

Aim of the present work is to develop a disposable coating of implanted biomaterial (**Implant Disposable Antibacterial Coating, I.D.A.C.**). The device, based on a novel resorbable hydrogel, would act as a fast resorbable local delivery carrier of antibiofilm and antibacterial compounds. The active drug (antibiofilm and antibiotic agents) will be mixed at the time of the hydrogel application during surgery, allowing the correct choice for any given patient, reducing costs and improving storage life and versatility of use[1,2].

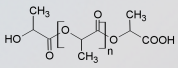
Rational

A novel polysaccharide based *graft* copolymer have been synthesized by grafting poly-lactic acid (PLA) chains onto a hyaluronic acid backbone; the resulting amphiphilic system is able to form physical hydrogels in acqueous media, due to the network that results from the association of the hydrophobic PLA chain.

Hyaluronic Acid (HA) is a biocompatible and biodegradable polysaccharide that is ubiquitous in the human body, clinically used for medical products for over three decades.

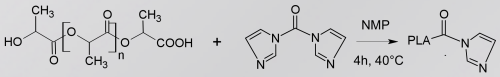


Poly-lactid Acid (PLA) is a biocompatible and biodegradable polymer, used in a wide range of medical applications. *In vivo*, PLA undergoes hydrolytic de-esterification to lactic acid, a normal metabolite of carbohydrate metabolism.

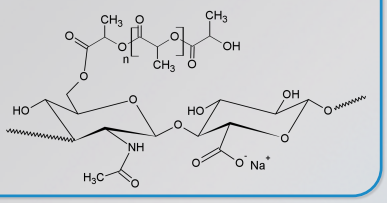


Synthesis of HA-g-PLA

In order to allow the grafting of PLA to HA, it is necessary to increase the reactivity of carboxylic group of PLA by preparing its imidazole derivative (PLA-CI).

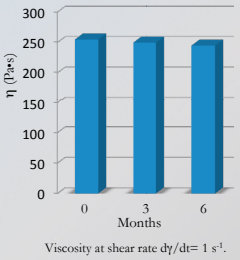


Subsequently, the reaction between carboxyl-activated PLA and an organic soluble HA form (HA-TBA) is carried out (NMP, 48h, 37°C); finally, precipitation and ion-exchange lead to the sodium salt form of HA-g-PLA.



Stability Tests

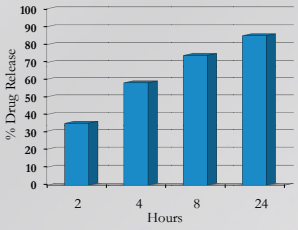
In acqueous media, PLA undergoes degradation to lactic acid, leading to a decrease of the hydrophobic interactions and, subsequently, to a decrease of the system viscosity. Stability tests were performed on different batches of I.D.A.C. hydrogels (HA-g-PLA 60 mg/ml in H_2O), by means of viscosity measurement and showed that system is stable at 4°C up to 6 months.



Viscosity at shear rate dγ/dt= 1 s^{-1}.

Release Tests

Release tests were performed in sink condition, placing the loaded hydrogel on a disk of titanium. Results show a rapid release of the antibiotic from the hydrogel within the 24 hours.



Vancomycin loaded I.D.A.C. was prepared with a polymer concentration of 6% w/v, and an antibiotic concentration of 2% w/v. A titanium disk (⌀ = 25 mm) was covered with 200 mg of hydrogel, and immersed at 37°C in 6 ml of Dulbecco Phosphate Buffer. Aliquots of the solution (1 mL) were taken at regular time intervals and replaced by an equal volume of fresh buffer

Safety Tests

In Vitro:
I.D.A.C. is not citotoxic[3] nor genotoxic[4].

In Vivo:
- The product does not cause allergic reaction[5], the skin irritation is irrelevant.
- After the subcutaneous application of the hydrogel, no adverse physical symptoms were observed, both in acute (4÷72 hrs) and subacute/subchronic toxicity (4 week)[6].
-After implantation in the rabbit femoral intramedullary canal, the product does not cause inflammatory reactions and/or degenerative processes in the bone tissue and in the intramedullary canal. No inflammatory signs or articular surfaces degeneration were observed. Histological investigations - after 12 weeks from the implant - have shown the absence of structural and morphometric alterations[7].

Efficacy

Nail implant in intramedullary rabbit femur – Procedure[8]

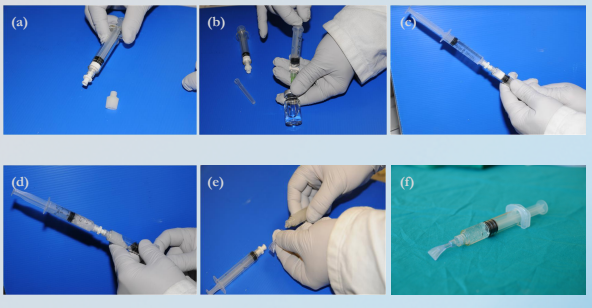
- Vancomycin loaded I.D.A.C. was applied on the nail.
- A hole of 3.5 mm was realized, and bacteria MRSA (Methicillin Resistant Staphylococcus aureus) were inoculated.
- The nail coated with I.D.A.C. was implanted.
- After 7 days, the rabbits were sacrificated.

The application of the hydrogel loaded with the vancomycin reduces the local bacterial load to 7 days from contamination even at high initial dose (10^6 cfu): the reduction is at least 99.95% when compared with the standard measures of prevention, or systemic administration of the antibiotic.

	Percentage of Reduction		
Vancomycin conc. in I.D.A.C.	Swap	Bone	Nail
2 % w/v	99.95 %	99.95 %	99.96 %
5 % w/v	99.96 %	99.95 %	99.96 %

Preparation Procedure

a) I.D.A.C. powder is in the syringe; on the syringe is inserted the luer-lock connector.
b) The sterile syringe is filled with the aqueous solution containing the antibiotic.
c) The two syringes are connected, and the component are mixed.
d) The formation of the gel takes place by steps repeated between the two syringes.
e) After complete hydration, the syringes are separated and the spreader is connected.
f) I.D.A.C. hydrogel (polymer concentration c_p= 60 mg/ml) is ready.



Conclusions

A novel HA-g-PLA copolymer have been synthesized, able to hydrophobically associate in an aqueous medium. The resulting hydrogel can be expolited in orthopaedic surgery: this material, loaded with the antibiotic vancomycin, was tested as a potential Implant Disposable Antibacterial Coating (I.D.A.C.): tests *in vitro* confirmed its non-cytotoxicity and non-genotoxicity; tests *in vivo* showed that the material causes no inflammatory reactions and/or degenerative processes in the bone tissue and in the intramedullary canal.
Vancomycin loaded I.D.AC.., tested for efficacy studies on nail implant in the intramedullary rabbit femur, was highly effective to inhibit the development of an infection even after a huge contamination: after 7 days from the contamination, the reduction due to the application of the hydrogel is at least 99% when compared with the standard measure of prevention.

Bibliography

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8) Orthopaedic Institute Rizzoli – Bologna (Italy)

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