Enhancing contrast agents and radiotracers performance through hyaluronic acid-coating in neuroradiology and nuclear medicine

Abstract
The use of hyaluronic acid nanoshells has been proposed to encapsulate prodrugs and exploit the mechanisms of interactions between living cells, like endocytes or cancer cells and hyaluronic acid, which is a natural component of the extracellular matrix. In this review we describe the potential and the limits of this promising research trend and discuss the theoretical advantages of such an engineering approach. Is it a possible scalability to increase the efficacy and biodegradability of molecules like contrast media and radiotracers especially for neuroradiology and nuclear medicine studies.

Introduction
One of the rapidly growing research fields referring to conventional and high-Tesla magnetic resonance imaging (MRI) and nuclear medicine studies is the study of new contrast agents and radiotracers capable to early detect microaggregates of cancer cells and cancer recurrence. Creation and development of solid tumors, of circulating tumor cells (CTC) and of early metastases may thus be a reality in the future.

Interaction between hyaluronic acid nanoparticles and living cells
Since hyaluronic acid (HA) has immunoneutrality, others [2, 4] and we [3, 5], proposed it as a biocompatible and biodegradable material for tissue engineering and for the development of delivery of various drug systems. Recently, formulations of several drugs or prodrugs conjugated to polymeric coated HA nanoparticles of poly(ε-caprolactone), polylactide, poly(lactic-co-glycolic acid), polyethylene–glycol, polycarylates and chitosan were found effective as smart delivery systems both in vitro and in vivo [5–9].

Hyaluronic acid is a natural linear polysaccharide constituted by repeating units of N-acetyl-D-glucosamine and D-glucuronic acid with monosaccharides and linked together by alternating β-1,3 and β-1,4 glycosidic bonds. The carboxyl groups of HA are predominantly ionized at pH 7.4 and therefore in physiological conditions, HA appears as a polyanion, known as hyaluronan [10]. Hyaluronic acid is found in a wide range of molecular weights ranging from 20kDa of the HA oligomers (o-HA), to the high-molecular weight (HMW) of bulk HA (~1.5MDa). In solution, the chains of HA adopt a random coil conformation, and its high hydrophilic nature leads to multiple hydrogen bonds with H2O, explaining the viscous and elastic characteristics of the connective tissues in which this polysaccharide is abundant. Besides chemical conjugation, it has been proved that HA can also be linked to other prodrugs or to proper delivery systems by weak interactions such as those involved in the formation of ion pairs [11, 12] expanding the number of possible candidates for conjugation with or encapsulation within those nanocarriers.

Hyaluronic acid conjugates could leverage on their propensity to overcome the blood brain barrier (BBB), so that first to produce biologic effects on the central nervous system (CNS) and secondly to provide a specific tumor targeting activity, which takes advantage of the peculiar interaction between HA receptors on the bilipidic membrane of glioma cells and the enzymes for HA degradation contained in the extracellular matrix (ECM). The first, is mainly based on receptor-mediated endocytosis of HA nanoparticles at the level of brain capillary endothelial cells [13]. The second, represents the basis for...
the matrix metalloproteinases (MMP)-triggered release of contrast agents in proximity to cancer cells aggregates [14, 15]. So, HA can be useful in neuroradiology and neuroncology. (Figure 1. Artistic representation of a CD44 transmembrane receptor).

**Technical advancements, potentials and limits in neuroradiology**

As anticipated above, HA nanoparticles as carriers for anti-neoplastic drugs hold the potential for an innovative, high throughput therapeutic approach in neuro-oncological chemotherapy protocols. They are used as innovative nanocarriers in order to provide stability and tolerance to molecules of contrast agents for MRI and other applications.

Hyaluronic acid seems to be used with complex manganese oxide nanoparticles (chemical formula: manganium oxide (MnO₃), Molar mass 228.812g/mol) with potential for selective accumulation within high grade gliomas.

The use of targeted tumor MRI in vivo has been so far mostly tested with polyethylene-glycol. Luo et al. (1999) conjugated MnO₃ with polyethylene-glycol creating nanoparticles of a mean diameter of 8.0nm and characterized by a good water-dispersibility, colloidal stability, cyto-compatibility and hemo-compatibility [16].

Chen et al. (2015) further investigated the applicability of MnO-polypeethylene-glycol nanoparticles conjugated with fluorescent dye cyanine5.5 as a dual-model imaging nanoprobe for MRI and for near infrared fluorescence. The dual potential imaging role of those nanoparticles was tested conducting experiments on the detection of brain gliomas in mice, showing both in vivo and ex vivo a preferential accumulation of those nanoprobes in the region of tumor cells [17].

Initial studies on HA nanoparticles are ruling out many of the concerns related not only to drug tolerance, but also those related to the risk of cytotoxicity and genotoxicity which have strongly affected the clinical testing of other drugs with a previously unremarkable laboratory track [18]. For instance, encouraging results in terms of cytotoxicity came from tests conducted on engineered nanoparticles synthesized by the encapsulation of polyethylene-glycol phospholipid shell around the MnO₃ core. By quantifying the induction of reactive oxygen species in human glioblastoma and neuroblastoma cell lines, Choi et al. (2015) demonstrated that the cytotoxicity of these nanoparticles were not significant and confirmed their high potential as an innovative diagnostic tool in CNS tumors [19].

**Externalities of HA nanoparticles in Nuclear Medicine**

Due to the extraordinary high sensitivity (down to the picomolar level of HA) and to its quantitative nature, radionuclide-based imaging is considered a standard modality for molecular imaging, although burdened by the poor resolution (≈5mm) of both proton emission tomography (PET) and single photon emission tomography (SPET) [20]. According to Lopci et al. (2015), in selected neuro-oncological cases, the use of PET scans can determine a change in treatment management in up to 50% of the cases, and these performances have further supported the optimization of known tracers or the identification of new ones [21]. A major challenge in the field of nuclear medicine is, for example, to develop disease specific nanoprobes with facile and robust radiolabeling strategies for gliomas. The characteristics needed are: imaging stability, improved targeting for elevated efficacy, enhanced sensitivity to detect tumors in their early stages, optimized in vivo pharmacokinetics for reduced non-specific organs uptake and reduced toxicity. As such, scientists have tried to understand whether the process of HA-coating could be of help in meeting those needs. (Figure 2. Structure of HA and its targets for chemical modification)

Over the past decade, several positron emitting labeled nanoparticles were developed and substantially improved to meet the diagnostic needs of a wide range of pathological conditions, including inflammatory and oncology ones. Specifically, the rationale behind the attempts to encapsulate radiotracers within HA nanoshells is to leverage on the elective interaction with CD44 and specifically increase the vehiculation within the CNS, while reducing the accumulation elsewhere, and favoring their release in the ECM adjacent to glioma cells. Initial experiments to label HA with indium-11, iodine-125, technetium-99m or carbon-14 (¹⁴C) radionuclides date back to the end of last decade [29, 30]. Cozikova et al. (2010) tested in a laboratory setting different
methods for the labeling of HA with routinely used radio-
nuclides aiming to compare the kinetic stability of radiola-
beled HA under different conditions (i.e. to mimic the inte-
raction with saline fluids, gastric juice and plasma following
intravenous or oral administration); they concluded that the
most suitable labeling method may vary from one radionu-
cleide to another, and depends on the specific characteristics
of the radiotracer tested.

To date, the main limitation to the HA-coating process has
been that the decay of the radiotracers mentioned above is
comprised between few minutes and 1 hour, although pre-
vious experiments with "Tc-labeled HA and fluorodeoxyglu-
cose-radiolabeled long-circulating polyethylene-glycol-
coated liposomes showed that they could remain in blood
circulation at near constant levels for at least 90 minutes [30,
31]. It is well known that the selection of appropriate radio-
nuclides depends on their imaging characteristics, decay
half-life, chelating properties, chemistry and availability; for
this, the radionuclides selected more recently for conjuga-
tion with nanoparticles, such as cuprum-64, bromium-76,
zirconium-89 and others who are characterized by longer
half-lives [32, 33].

At present, despite the enthusiasm around HA-coated
radiotracers, no one has gone beyond phase II in clinical tri-
als; despite their clinical use is not imminent further inves-
tigations are certainly warranted.

In conclusion, the pace of effective translation from bench
to bedside of the latest basic science achievements des-
cribed in this short review article is meant to accelerate even
further in the next decade, this will most likely lead to an
optimization of the diagnostic tools available for those pat-
thologies, and will potentially support a shift towards perso-
nalized medicine for our patients.

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