In vivo imaging of the fate of a hybrid nanoparticulate BMP-7 drug delivery system after injection to a mouse model of distraction osteogenesis

Nayef L., Hamdy R.* and Tabrizian M.*
Genome Lab, McGill Univ, Biomed Eng, Montreal Canada (lamees.nayef@mail.mcgill.ca)

INTRODUCTION AND OBJECTIVE

Bone tissue regeneration is needed not only by people who suffer from developmental problems that cause them to have discrepancies in limb lengths, but also by many that loose significant volumes of bone through accidents, infections or during the treatment of bone malignancies. Distraction Osteogenesis is a procedure that allows the regeneration of bone inside the body from scratch, avoiding problems of disease transmission, immune rejections and need for secondary surgeries connected with implanting bone grafts. It works by cutting the bone to be lengthened into two pieces and holding the pieces apart using a mechanical fixator that is adjusted each day to increase the length of the gap between the two bone pieces (Haidar Z. 2009). This mechanical strain accelerates reactions in the body that cause bone growth allowing new bone to fill the full length of the distraction gap. Despite its many advantages distraction osteogenesis has one major limitation: the length of time it takes for completion. Patients must wait 6-7 months for a bone growth of 5 centimetres, all while wearing mechanical fixators that hinder their daily activities and increase risks of infections (Haidar Z. 2009). Injecting exogenous supplies of growth factors can speed up the process (Chen D. 2004); however, the high diffusivity of these growth factors causes them to be lost to other tissue, causing bone growth at unwanted sites and less healing than desired (Haidar Z. 2009). A drug delivery system is needed to allow the efficient and safer use of growth factors. We designed a drug delivery system that releases BMP-7, a potent growth factor which accelerates bone formation. Our design consists of a nanoparticulate hybrid system, with a liposomal core and six coating alternating layers of alginate and chitosan (Figure 1). BMP-7 is encapsulated in the liposomal core and between the layers and is released slowly as the layers biodegrade (Haidar Z. 2008). The work done by our group proved the system to be biocompatible and functional (Haidar Z. 2010). However, the efficiency of the design cannot be fully determined unless the path that the injected drug delivery system takes is known. Slow prolonged release is meaningless if the drug delivery system diffuses away from the distraction gap and releases its contents in undesired sites. This would cause unnecessary loss of expensive drug, less healing than desired at the distraction gap and more side effects to other tissues (Haidar Z. 2009).

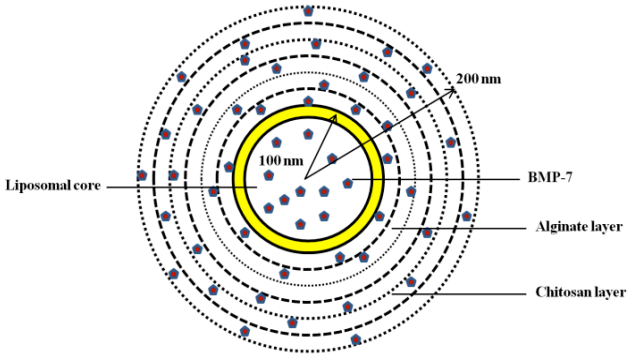

Figure 1: Schematic of our drug delivery system design with the liposomal core and 6 alternating layers

The objective of this paper is to verify the efficiency of the drug delivery system by following its fate in the body with time after injection. The liposomal core for the drug delivery device is used to encapsulate fluorescent quantum dots instead of BMP-7 to achieve the objective. The loaded liposomes are injected into the distraction gap of mice that underwent distraction osteogenesis then tracked by following the position of fluorescence emissions from quantum dots with time.

MATERIALS AND METHODS

Materials

1, 2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC; CordenPharma, Switzerland), cholesterol (Sigma Aldrich, US) and dimethyldioctadecyl-ammonium bromide (DDAB; Sigma, US), chloroform and methanol (Fisher Scientific, New Jersey, US) were used to make liposomes. Carboxyl conjugated quantum dots with emissions centered at 700 nm (trade name eFluor@700 NC) were purchased from eBioscience. Sephadex G-50 (Sigma Aldrich, US) and 19 mm, polycarbonate, 200 nm pore size filters (Whatman, US) were also used.

Incorporating quantum dots into liposomes

Liposomes were made using the dry thin film hydration method by dissolving 100 mg of DPPC, 26.3 mg of cholesterol and 5.15 mg of DDAB in 4:1(V/V) solution of chloroform and methanol respectively. The mixture was dried using roto evaporation under a vacuum and the resulting thin film hydrated with a 670 pM solution of quantum dots in Milli-Q water. The resulting mixture was extruded 11 times through two polycarbonate filters with a pore size of 200 nm to make unilamellar 225 +/- 0.7 nm sized liposomes, with a zeta potential of 25.93+/-1.08

nm (same characteristics as the liposomal core of our drug delivery system).

Separating loaded liposomes from un-encapsulated free quantum dots

The extruded mixture was passed through Sephadex G-50 in a chromatography column to separate quantum dot loaded liposomes from free unencapsulated quantum dots.

Distraction Osteogenesis and injection of mice

Distraction osteogenesis was performed on eight mice. Six mice were injected at the distraction gap with 0.01 to 0.02 mL of the purified quantum dot loaded liposomes. The injected mice were divided into three groups of two with each group sacrificed after 1, 24 or 48 hrs of injection. The un-injected mice were sacrificed and used as controls.

In vivo Imaging of mice

The eXplore Optix MX2 preclinical optical imaging device was used to image mice after their sacrifice. The device excited the specimens using a 470 nm emitting laser. Fluorescent emissions at wavelength 693 nm or higher were collected from each excited volume in mice's bodies. The emissions of the injected mice were compared with the emissions from the control un-injected mice to differentiate between natural background fluorescence emissions and emissions due to the injected quantum dot loaded liposomes. An X-ray image of the distraction gap was also taken.

RESULTS AND DISCUSSION

The quantum dots used were chosen to emit at 700 nm because infrared emissions are not absorbed easily by tissues in the body allowing better detection of the emissions even under several layers of tissues (Sandros M. 2007). Imaging of the mice showed that a higher than background fluorescence emission is noted in the right leg of all injected mice at the same site as the injection. An x-ray image confirmed that these higher emissions are coming exactly from the same place as where the distraction gap was present (Figure 2). There was no difference in the location of these emissions after 1 hr, 24, or 48 hours of injection (not all images are shown). This shows that the quantum dot loaded liposomes stay at the distraction gap for at least 48 hours after injection (Figure 2). Our fully coated drug delivery system (400 nm) is heavier than the 200 nm liposomal core and is very likely to stay at the distraction gap as well. Staying at the distraction gap ensures that BMP-7 released from our drug delivery system is used optimally to make bone at the desired site.

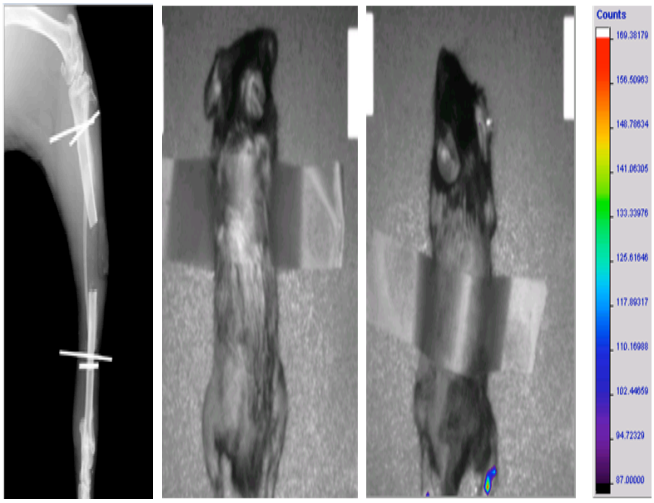

Figure 2: (Left) X-radiograph of one of the injected mice; (middle) emissions from control mouse, (right) and a mouse injected and sacrificed after 48 hours. The highest emissions are noted at the injection site (i.e distraction gap).

CONCLUSIONS

Our nanoparticulate drug delivery system is very efficient at drug delivery: it does not diffuse away to locations other than the distraction gap for at least 48 hours, allowing bone growth to occur optimally at the desired site. The localization of drug release also minimizes unnecessary losses of the drug that can cause side effects and undesired bone growth.

REFERENCES

- Chen D. et al. (2004) *Bone morphogenetic proteins.* Growth Factors 22 233-241.
- Haidar Z. et al. (2008) *Protein release kinetics for core-shell hybrid nanoparticles based on the layer-by-layer assembly of alginate and chitosan on liposomes*. Biomaterials 29 1207-1215.
- Haidar Z. et al. (2009) *Modulated release of OP-1 and enhanced preosteoblast differentiation using a core-shell nanoparticulate system*. Journal of Biomedical Materials Research Part A 91A 919-928.
- Haidar Z. et al. (2010) *Biocompatibility and Safety of an Injectable Hybrid Nanoparticulate rhOP-1 Delivery System intramuscularly administered in Rats*. Biomaterials 31 2746-2754.
- Sandros M. et al. (2007) *InGaP@ZnS-Enriched Chitosan Nanoparticles: A Versatile Fluorescent Probe for Deep-Tissue Imaging*. Advanced Functional Materials 17 (18) 3724-3730.

ACKNOWLEDGMENTS

The authors thank NSERC and CIHR for supporting this research. The authors also thank Dr. Abdallah Husseini for his assistance in performing distraction osteogenesis and injections to the mice.